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Review Article

Medicinal Plant Extracts as Process-Dependent Bioactive Systems for Aquaculture: Phytochemistry, Extraction Engineering, Stability and Exposure

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Abstract *- Medicinal-plant products have been investigated in aquaculture as feed additives, immunomodulators and agents for pathogen control, but studies of the same plant species often report different outcomes. Some of this variation reflects differences among the materials tested. Biomass source, extraction procedure, downstream processing and route of administration can each alter extract composition and exposure. This critical narrative review considers aquaculture evidence together with studies of extraction, stability and formulation. Each preparation is treated as a manufactured chemical mixture rather than as a fixed property of the source plant. The evidence map separates assays conducted directly on pathogens from experiments in aquatic hosts and records whether the tested preparation, administered dose and delivered exposure were characterized. Across the reviewed studies, crude-extract yield was not a reliable measure of potency because solvent composition, contact conditions, mass transfer, concentration and drying can affect constituent recovery and degradation simultaneously. Preparations assigned to the same broad phytochemical class may also differ in substitution, glycosylation, ionization, molecular size, volatility and sulfur reactivity. Feeding and challenge experiments reported beneficial, neutral and adverse responses, often with marked dose dependence. Storage, release, dilution, sorption, uptake and metabolism can further alter the material between product addition and biological action. Evidence from extraction and life-cycle studies likewise does not support the assumption that natural origin, aqueous processing or shorter treatment necessarily confers environmental benefit. A staged development scheme is therefore proposed in which batch specifications, route-specific exposure, efficacy, toxicity and pilot-scale performance are established before commercial use is considered.*

Keywords *- Aquafeed delivery, Botanical standardization, Dose–exposure relationship, Mass transfer, Phytogenic products, Process sustainability.*

1. Introduction

Aquatic foods make an important contribution to human nutrition. A recent FAO estimate places the combined production of aquatic animals and algae at 235 million tonnes, with aquaculture accounting for much of the continuing increase. This aggregate includes capture output and algal production and should not be interpreted as the quantity of farmed fish. [1] As production becomes more intensive, the consequences of crowding, inadequate feed, unstable water quality and infectious disease become more severe. Handling and environmental stress can impair host defense and increase losses from opportunistic infections.

Against this background, medicinal plants have been investigated as dietary stimulants, appetite modifiers, stress-management additives and sources of antipathogenic activity in fish and shrimp. [2, 3] Plant-derived products have a supporting role in aquaculture health management. They do not replace sound biosecurity, adequate nutrition, vaccination where available, or treatment based on diagnosis. Nor does a "natural" or chemically complex origin establish that a preparation is safe, inexpensive or environmentally benign. Activity measured in the laboratory may be reduced or lost during pelleting, storage, dispersion in culture water or digestion. Evaluation must therefore consider the composition tested and the response measured, rather than the botanical name alone. [4, 5]

A botanical extract is not defined by species identity alone. Plant organ, harvest condition, moisture, solvent, extraction time and finishing steps influence which compounds are recovered and retained. Molecular structure affects

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solvation, phase partitioning, redox chemistry and susceptibility to reaction, whereas equipment and operating conditions control recovery and loss. Preparations made from the same species may consequently represent different experimental materials. [6, 7]

The relevant literature often separates questions that need to be considered together. Aquaculture syntheses commonly organize evidence by candidate plant or biological endpoint, whereas extraction and phytochemical studies may end after measuring yield, composition or an in vitro response. It is then difficult to connect reported bioactivity with the production, storage and administration of a reproducible aquaculture input.

Grouping evidence by plant can also obscure differences among fractions, carriers and routes of administration. The scope of this review is restricted to live-animal applications; post-harvest preservation is considered only where its chemistry helps explain the behavior of a preparation before administration. [2, 3, 7, 8]

This review, therefore, uses the preparation, rather than the species name, as its organizing unit. Evidence from efficacy studies is examined alongside information on starting biomass, processing, composition, stability and exposure. The central questions are whether a defined material works under stated conditions and whether its manufacture, delivery and safety have been characterized sufficiently for further development.

Study mapping, process-based models, numerical case comparisons and a sequence of development gates are used to distinguish reported observations from assumptions. The article presents neither a new efficacy experiment nor a pooled treatment estimate.

2. Literature Search and Review Methodology

2.1. Scope and Source Identification

This article is a critical narrative review, not a systematic review or meta-analysis. An existing compilation of aquaculture literature was supplemented with chemical, biological and engineering sources. During September 2026, targeted searches and checks of relevant articles were used to clarify preparation identities, verify numerical claims and address specific gaps in extraction, stability and environmental interpretation. Sources included publisher pages, PubMed records and accessible repository copies. Scopus and Web of Science were not searched, so the resulting literature set is not an exhaustive census.

The discovery query combined ("plant extract" OR phytochemical), (aquaculture OR fish OR shrimp), biological end-points and terms related to extraction or stability. Subsequent exact-title, DOI and focused process searches were used to verify individual claims and fill defined thematic gaps. Source discovery, primary-source verification, evidence coding and synthesis were treated as separate steps. Screening counts were not assigned retrospectively because they had not been logged prospectively.

2.2. Eligibility and Evidence Boundaries

Application studies were eligible when they examined medicinal plant material, extracts, fractions or isolated plant-derived compounds in relation to aquatic animals, aquaculture pathogens, growth, immune responses, physiological condition or toxicity. Mechanistic and engineering studies from outside aquaculture were included only when they directly informed extraction selectivity, chemical transformation or formulation.

Such studies are treated as transferable process or chemical evidence, not as evidence of efficacy in fish. Seaweed preparations were retained as comparative aquatic biomass systems where they clarified chemistry or delivery; their inclusion does not classify all seaweeds as medicinal terrestrial plants.

Unrelated terrestrial efficacy studies, plant-virus experiments without an aquatic target, duplicate reports of the same experiment and poorly specified commercial claims were not used to establish aquatic efficacy. Propolis and mushroom products were not classified as medicinal plants. Reviews provided context and helped identify relevant studies, but named experimental results were attributed to the primary study wherever it was accessible.

When only an abstract was available, details of dose, route and outcome were not reconstructed from practices considered typical for that plant. Older evidence was retained when informative; historical recognition, however, was not treated as validation of a currently marketed product. [9]

2.3. Extraction, Evidence Coding and Limitations

Each study was considered along the following sequence: plant identity → part → extraction method and solvent → process conditions → chemical profile → host/target → dose and route → biological response → stability/formulation → limit of in-interpretation. Table 2 applies this framework through linked study IDs. "Not Reported" (NR) was distinguished from "Not Verified" (NV), preventing inaccessible information from being classified automatically as poor reporting.

Direct assays (D) comprised microbial inhibition, parasite assays and infected-cell experiments. Host experiments (H) comprised feeding, injection, immersion, challenge and toxicity studies. The designation D+H records the presence of both forms of evidence; it does not imply that a molecular mechanism has been demonstrated. Production-scale evidence would also require a documented production setting, comparator and replication. Use of the term "large-scale" in a title was not sufficient. Chemical identification, quantified

marker delivery and measured exposure were assessed separately from the biological endpoint. The labels describe the questions a study can address and are not a validated numerical quality score or certainty-of-evidence rating.

During synthesis, direct pathogen inhibition was separated from host-mediated resistance, and biomarker changes were distinguished from production or clinical benefit. Better performance of a mixture relative to an untreated control was not accepted as evidence of synergy. Verified numerical results retain their units, route, duration and comparator. Equations used in the review are analytical constructs rather than models calibrated across the reviewed trials.

2.4. Structured Risk-of-Bias and Reporting Appraisal

The appraisal covered six domains: treatment allocation and experimental-unit independence; comparator suitability; blinding or objective outcome ascertainment; attrition and completeness of outcome reporting; chemical definition of the tested preparation; and correspondence between nominal dose and measured exposure. These domains were applied according to study design and were not collapsed into a numerical score. Direct microbial assays, feeding trials, injection studies and process experiments do not share a single validated risk-of-bias tool. Table 1 consolidates the recurring concerns and minimum evidence requirements.

The limitations differed among study designs. Tank-level randomization, blinding and attrition handling were often not verifiable in host trials. Many direct assays identified the solvent fraction more clearly than the active chemical composition. Injection studies could support hypotheses about host responses, but their relevance to feed or bath application was limited by the different exposure route.

Process studies generally measured extraction outcomes more rigorously than aquaculture benefit, whereas challenge studies seldom established a quantitative balance for marker delivery or exposure. Positive, null and adverse findings were retained to reduce directional selection, although inaccessible methods and selectively reported endpoints remain sources of uncertainty.

2.5. Quantitative Comparability and Decision Not to Pool

A pooled effect estimate was not calculated. The evidence includes crude powders, solvent extracts, essential oils, mixtures, polysaccharides and isolated compounds administered through incompatible routes and dose denominators. Growth, minimum inhibitory concentration, challenge survival, immune markers and parasite burden also cannot be converted to a single defensible outcome scale. Pooling would therefore create numerical precision without chemical or biological comparability. Quantitative synthesis was restricted to within-study contrasts and cross-case ratios with explicit denominators and endpoints.

3. Literature Landscape, and Evidence Mapping

The evidence spans growth and feed use, antioxidant and immune responses, antibacterial, antiviral, anti-oomycete and antiparasitic applications, together with extraction, stability, formulation and sustainability studies. Its most informative distinction is how far a preparation has been followed from manufacture to measured exposure. Crude extracts, mixtures, essential oils, polysaccharides and isolated compounds cannot be pooled by botanical name without discarding variables governing reproducibility. Table 2, therefore, retains favorable, null and adverse findings and separates preparation information from exposure and outcome data.

Figure 1 illustrates the coverage of endpoints within the subset that was directly evaluated, rather than indicating effect size or bibliometric strength. Accordingly, *Artemisia* is included as evidence of growth despite its unfavorable outcome, whereas *Moringa* represents a counterexample concerning safety. [10,11] Studies for which the source identity could not be reliably resolved were excluded to avoid assigning them an unwarranted level of evidentiary strength.

Grouping studies by plant name obscures several important differences among the tested preparations. Solvent comparisons for *Andrographis* and in the Chinese anti-oomycete screen show directly that the measured activity depends on how the extract was prepared. [12, 13] In *Macleaya*, bioassay-guided fractionation linked the observed activity to sanguinarine, whereas the active constituents remain unresolved in most studies of crude extracts. [14] The garlic study also measured leaching from the feed, providing information on exposure after formulation. By comparison, injection studies using *Toona* and *Tinospora* bypassed the uptake barriers associated with ingestion. [15–17] Quantitative contrasts reinforce the same point. In the *Quil-laja* feeding study, weight gain at 150 mg/kg feed was 372% of starting mass versus 327% in controls, an absolute difference of 45 percentage points and a relative difference of approxi-mately 13.8%; the 300 mg/kg group reached 325%, showing no monotonic dose response. [18] In the anti-oomycete screen, the *Aucklandia lappa* fraction had EC_{50} values of 11.3 mg/L for *Saprolegnia* and 26.1 mg/L for *Achlya*, a 2.31-fold end-point difference within the same preparation. [13] The aqueous *Andrographis* preparation produced an MIC of 31.25 μg/mL in its specific bacterial assay, but that concentration cannot be converted into a feed or immersion recommendation. [12] These cases provide quantitative guidance while preserving the preparation, organism, route and endpoint attached to each value.

4. Phytochemical Composition and Chemical Functionality

4.1. Phenolics, Flavonoids, and Tannins

Phenolics are defined by an aromatic ring bearing one or more hydroxyl groups, but a bulk phenolic assay gives little

information about the behaviour of the individual molecules in an extract. Most flavonoids share a C6–C3–C6 arrangement, with two aromatic systems connected through an oxygenated ring.

Differences in unsaturation, oxidation, substitution and stereochemistry distinguish flavonols, flavones, flavanones and flavan-3-ols. These structural features alter hydrogen bonding, charge delocalization and steric access to reactive positions. Extracts with comparable assay totals may therefore differ substantially in chemical behavior. [6, 7]

Quercetin, reported in *Phyllanthus*, carries hydroxyl substituents at 3, 5, 7, 3′ and 4′. Its catechol arrangement on the B ring, together with conjugation across C2=C3 and the 4-carbonyl, favours radical stabilization. Removal of the 3′ hydroxyl gives the substitution pattern of kaempferol, whereas saturation of C2=C3 distinguishes a flavanone such as naringenin. These comparisons identify features of chemical relevance, but they do not support a universal ranking of activity across solvents, tissues or assays. Glycosylation also needs to be reported because glycosides and aglycones differ in size, hydration and susceptibility to enzymatic conversion. [19]

ID	Plant / preparation	Bacteria	Viruses	Oomycetes	Parasites	Immunity	Growth
E01	*Andrographis paniculata*	D+H	—	—	—	—	—
E02	*Persicaria odorata*	D+H	—	—	—	H	H
E03	GF-7 mixture	D+H	—	—	—	H	—
E04	*Olea europaea*	—	D	—	—	—	—
E05	Indian plant screen	—	H	—	—	—	—
E06	Cynodon dactylon	—	H	—	—	—	—
E07	Three Chinese medicinal plants	—	—	D	—	—	—
E09	*Santalum album*	—	—	—	H	—	—
E10	*Allium sativum*	—	—	—	H	—	—
E11	*Macleaya cordata*	—	—	—	D+H	—	—
E12	*Ocimum sanctum*	H	—	—	—	H	—
E13	*Tinospura cordifolia*	H	—	—	—	H	—
E14	*Toona sinensis*	H	—	—	—	H	—
E15	*Astragalus* polysaccharides	—	—	—	—	H	H
E16	*Quillaja* saponins	—	—	—	—	—	H
E17	*Artemisia arborescens*	—	—	—	—	H	H
E18	*Moringa oleifera*	—	—	—	—	—	—
E19	Ginsana G115	—	—	—	—	—	H
E21	Curry / marigold leaves	—	—	H	—	H	—

D: direct assay H: host experiment D+H: both

Coverage, not potency or risk-of-bias ranking.
- = not mapped, not evidence of no effect.

Fig. 1 Selected-study evidence landscape. Study IDs are defined in Table 2. D: direct assay; H: host experiment; D+H: both. Color encodes design coverage, not benefit, magnitude or risk of bias

Phenolic acids contain a carboxyl group in addition to a hydroxylated aromatic system; caffeic acid also contains a conjugated side chain. Tannins cover a wider range of structures. Hydrolysable tannins contain ester-linked phenolic units, condensed tannins are formed from coupled flavan-3-ols, and brown-algal phlorotannins consist of phloroglucinol units connected by carbon–carbon or ether bonds. Molecular size and hydroxyl-group accessibility influence both radical reactions and protein binding. A phenolic-rich additive may therefore re- tard oxidation while also affecting protein availability or release from a carrier. [20]

Class-level detection of tannins or flavonoids provides only preliminary information about an aquaculture product.

Inter-pretation depends on knowing which constituents recur across batches, survive processing and remain available in an unbound form at the relevant site. Different chemotypes may occur within the same plant species, and extraction can introduce further variation in composition. Claims of efficacy should consequently be attributed to a chemically characterized preparation, not to a composition inferred retrospectively from the observed biological response.

4.2. Alkaloids, Terpenoids, and Organosulfur Compounds

Alkaloids include chemically diverse nitrogen-containing ring systems that may occur in different charge states. In members containing amine groups, protonation can substantially alter water solubility and phase distribution, although some alkaloids are not protonatable free bases. Berberine is a permanently charged protoberberine, while sanguinarine contains an iminium-bearing benzophenanthridine framework.

Their charge, heteroaromatic framework and oxygen substitution distinguish these compounds from neutral terpenoids. Activity reported for an individual alkaloid cannot establish a shared molecular target for the entire class. [7, 21]

Terpenoids are assembled from isoprenoid units and range from monoterpenes to substantially larger triterpenes. Essential oils, however, are volatile multicomponent mixtures. Thymol and carvacrol share phenolic hydroxyl, methyl and isopropyl substituents, but differences in the positions of these groups can change their intermolecular interactions. Menthol adds chirality and an alcohol group, while other oil constituents may include aldehydes, ketones, ethers or esters. The label "essential oil" therefore implies neither a fixed polarity nor a consistent biological response. [22]

Although andrographolide has a largely hydrophobic diterpenoid skeleton, it retains several polar functional groups. Its hydroxyl and lactone groups affect solvation, and its stereochemistry may influence interactions with biological targets.

Saponins achieve amphiphilicity through a different structure, in which a steroidal or triterpenoid aglycone is joined to one or more sugars. This arrangement favours interfacial effects but, on its own, provides no evidence of gill safety or benefit across feed inclusion levels. [6, 21]

For garlic, preparation initiates part of the chemistry relevant to the final product. Tissue disruption brings alliin into contact with alliinase and produces thiosulfinates such as allicin. The oxygenated sulfur–sulfur unit of allicin differs chemically from the more persistent sulfides formed after some types of processing. Crushing, heating, storage and formulation can alter both allicin formation and its subsequent loss. Fresh extract, powder and garlic oil are therefore not interchangeable sources of an "allicin dose." [4]

4.3. Analytical Identity and Batch Standardization

Polysaccharide-rich preparations, including products derived from *Astragalus*, require descriptors different from those used for small secondary metabolites. Monomer composition, linkage pattern, branching, molar-mass distribution and accompanying impurities may all affect function. Reporting a total polysaccharide percentage does not establish that the material is a single purified compound. Observed aquaculture responses justify including such preparations in the evidence base, but assignment of a molecular mechanism requires further structural characterization. [21, 23]

Analytical reports need to distinguish quantified markers from tentative annotations. HPLC with appropriate standards is suited to nonvolatile markers, whereas gas chromatography–mass spectrometry is commonly used for volatile fractions. Agreement among retention behavior, fragmentation and authentic standards increases confidence in an assignment; nuclear magnetic resonance can provide fuller structural resolution where required. A library match alone seldom distinguishes isomers or stereochemistry. For production purposes, specifications should report marker concentration in the dry extract and recovery relative to the starting biomass.

The *Persicaria odorata* feeding study linked chemical analysis with a host experiment. HPLC detected catechin and quercetin, while rutin was not detected under the stated conditions; radical-scavenging activity was measured separately. The design associates an analytically described extract with the host response, but does not show that either detected marker caused it. [24] In the largemouth bass experiments, the chromatographic profile of the GF-7 mixture likewise supports quality control of the mixture without attributing efficacy to an individual ingredient. [25]

Colorimetric totals can support rapid screening, provided that they are not treated as comprehensive compositional analyses. Reported total phenolic and flavonoid values represent assay responses relative to standards rather than molar quantities of active constituents. Batch-release specifications should additionally cover moisture, microbial quality, residual solvent, contaminants and changes during storage. Together, these measurements make it possible to identify the administered material and assess its comparability across experiments.

Phytochemical classes also overlap in chemically relevant ways. Tannins and flavonoids are phenolics, whereas thymol and carvacrol are terpenoids in origin but display phenolic functionality. Extraction behaviour cannot be inferred from polarity alone because ionization, association with the plant matrix and solvent access also affect recovery. Table 1 relates these chemical properties to process decisions without assigning a general ranking of potency to any class. [6, 20, 22]

5. Extraction Technologies and Process Variables

5.1. Solvent Selection and Process Objectives

Aquaculture studies have used water, alcohols, ethyl acetate and other solvents to prepare plant extracts. The resulting materials are not chemically equivalent. Recovery depends on polarity, hydrogen bonding and solute ionization, as well as on penetration of the solvent into the plant matrix. Water-rich mixtures tend to recover highly hydrated constituents, whereas less polar phases may concentrate lipophilic material. Changing the ethanol–water ratio alters extraction selectivity, although the appropriate composition varies with the plant material and the marker being measured. [7] Figure 2 shows the broader consequences of solvent choice and processing conditions.

The largest mass of dried solids is not necessarily the most useful process outcome. Additional yield may consist mainly of sugars, mineral material, pigments or other inactive co-extracts. For an aquafeed ingredient, marker recovery has to be considered alongside retained activity, impurity limits, residual-solvent control and cost. A solvent suitable for exploratory work may be unacceptable for production. If it is replaced, the resulting preparation requires chemical comparison and renewed biological validation.

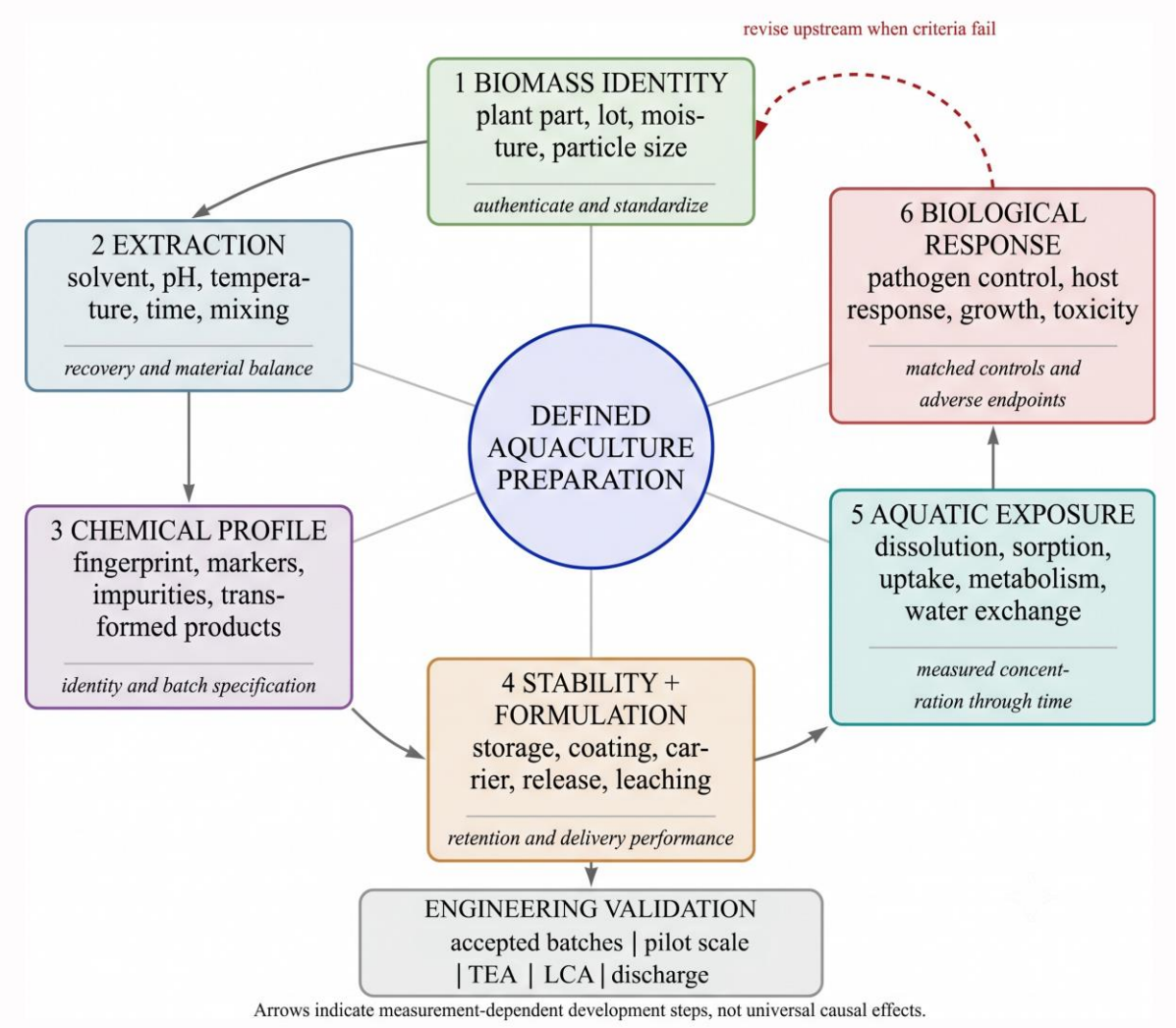


Fig. 2 Process–composition–activity framework proposed in this review. The preparation is followed from authenticated biomass through extraction, chemistry, stability, formulation and aquatic exposure to biological and engineering outcomes. Each right-hand box is a measurement or decision checkpoint. Connections represent research questions, not fitted causal relationships; unfavorable results require revision of the upstream process

A process account should track the starting dry biomass, liquid product, retained cake, solvent vapour and other losses separately. Although concentrating a dilute stream can simplify handling, it may expose the extract to heat or cause precipitation. Solvent recycling reduces fresh-solvent demand only if water content and accumulated contaminants are controlled, since changes in the recycled solvent can influence subsequent extractions. These are proposed design requirements, not operating specifications reported in the historical feeding studies.

5.2. Mass Transfer and Extraction Rate

Release from plant solids proceeds through solvent wetting, dissolution or desorption, movement within the particle and transport across the surrounding liquid film. Milling mainly increases the available surface area and shortens internal diffusion paths, whereas agitation more directly reduces resistance in the surrounding liquid film.

Either operation may shorten the time required to approach equilibrium, but neither changes the equilibrium solubility. Extraction rates cannot be generalized across plant materials because drying history, cellular structure and interactions with proteins or polysaccharides also influence release, especially when phenolics are distributed between soluble and bound fractions. [20]

Equation (1) gives a lumped batch balance for use in future experiments:

$$V_{\mathrm{L}} \frac{\mathrm{d}C}{\mathrm{d}t} = k_{\mathrm{L}} A (C^{*} - C) \qquad (1)$$

Here, V_{L} denotes liquid volume, C the bulk marker concentration, k_{L} an effective liquid-side mass-transfer coefficient, A the accessible interfacial area, and C^{*} the interfacial equilibrium concentration.

For volume in m^3, concentration in kg m^{-3}, area in m^2 and kL in m s^{-1}, both sides have units of kg s^{-1}. The Initial assumptions are constant volume, adequate bulk mixing and negligible chemical transformation; these conditions are not universal features of botanical extraction.

With a finite solid charge, C^{*} may change as the matrix is depleted, making a constant driving-force interpretation unreliable near exhaustion. Intraparticle diffusion introduces a characteristic timescale proportional to the square of particle length divided by effective diffusivity.

Smaller particles may accelerate recovery, but excessive milling can hinder filtration and increase the recovery of undesirable material. Greater agitation is most useful when external resistance is important; it will not necessarily overcome a predominantly intraparticle limitation.

A kinetic experiment requires a time series and the mass of marker recovered at each sampling point. A single terminal percentage cannot distinguish among release mechanisms. Early samples may be dominated by surface washing, followed by slower release from within the particles.

Models should be judged by residuals, parameter uncertainty and physical credibility as well as fit statistics. The aquaculture application literature provides no basis for assigning one extraction-rate constant to all medicinal plants.

5.3. Competing Release and Degradation

Higher temperature can improve solvation and transport while also accelerating oxidation, hydrolysis and volatilization. A process may consequently produce more dried extract but recover less of the desired parent compound. Product definition must consider temperature, pressure, solvent composition and residence time together. [7, 22]

For a finite releasable marker inventory, release and degradation can be represented by the simplified model:

$$\frac{\mathrm{d}m_{\mathrm{s}}}{\mathrm{d}t} = -k_{\mathrm{rel}} m_{\mathrm{s}},$$

$$\frac{\mathrm{d}m_{\mathrm{l}}}{\mathrm{d}t} = k_{\mathrm{rel}} m_{\mathrm{s}} - k_{\mathrm{deg}} m_{\mathrm{l}}. \qquad (2)$$

From a process perspective, extraction should stop when degradation and resource use outweigh the marginal recovery. Common thermodynamic or kinetic parameters were not estimated because the available aquaculture studies do not provide a compatible dataset.

5.4. Ratios, pH and Process Comparability

The solid-to-liquid ratio is informative only when calculated against a defined mass of dry biomass. A larger solvent volume may improve wetting of the plant matrix and delay saturation, but it also produces a more dilute extract and increases the solvent-removal requirement. Using less solvent creates different difficulties. Concentrated slurries can become viscous or remain incompletely wetted, which hinders mixing, while solvent retained in the filter cake may represent a substantial loss of recovered material. Process comparisons should include both the initial solvent charge and the volume used for washing; without these values, a higher reported yield may reflect greater total solvent use. Fresh and dried materials must also be compared on a dry-mass basis because equal fresh masses contain different amounts of dry solids and markers.

Changes in pH affect extraction selectivity and can also place some constituents under chemical stress. Ionization may increase apparent aqueous solubility while decreasing partitioning into a nonpolar phase, but this change does not necessarily improve transport across membranes. Strongly acidic or alkaline conditions can transform glycosides, lactones and other susceptible compounds, and subsequent neutralization cannot restore the original chemical profile.

The pH-dependent persistence of allicin and the different precipitation and degradation behavior of curcumin show why no single general pH optimum can be assigned to a mixed extract. [8, 26] The pH should be measured during extraction, after concentration and after addition of the preparation to the assay medium or culture water. Without these measurements,

antimicrobial activity resulting from a change in medium pH may be misattributed to the extract.

The effects of agitation and particle-size reduction depend Here m_s is the releasable marker mass remaining in the solid, m_l is the undegraded marker in the liquid, and k_{rel} and k_{deg} are conditional first-order coefficients. The model assumes one-way release, no solubility limit, negligible degradation in the solid phase and a well-mixed liquid. Reversible sorption, precipitation and equilibrium limitation would require additional terms. With these assumptions, the recovered parent mass may reach a maximum while crude solids continue to accumulate. Gravimetric extraction yield is not a thermodynamic equilibrium constant, and a temperature-related increase in yield does not establish that the transfer process is endothermic. Where possible, solid-phase release and disappearance from the liquid phase should be measured in separate experiments. Applying an Arrhenius treatment to an apparent coefficient is defensible only when the controlling regime remains the same across temperatures. The fitted activation energy should not then be assumed to represent a molecular reaction barrier. From on the resistance that controls extraction. Stronger mixing can reduce resistance in the external liquid film, but it cannot compensate for slow diffusion within plant particles. Intensive milling may increase early marker release, although the resulting fines can reduce filtration throughput. Process comparisons should report suspension quality, filtration time, marker remaining in the wet solids and total processing time. Revolutions per minute alone provide an inadequate basis for scale-up because performance also depends on impeller geometry, power input, slurry rheology and mixing time. These are proposed design considerations, not operating specifications derived from the historical feeding trials.

5.5. Recovery, Purity and Activity as Different Objectives

Three measurements help distinguish productive recovery from the production of a large mass of dilute solids:

$$Y_{ext} = \frac{m_{ext,dry}}{m_{biomass,dry}},$$

$$W_{marker} = \frac{m_{marker,ext}}{m_{ext,dry}}$$

$$R_{marker} = \frac{marker,ext}{m_{marker,initial}} \quad (3)$$

All three ratios are dimensionless. Added carriers are excluded from the dry-extract mass, and the initial marker inventory must be measured using a validated reference extraction rather than assumed. Co-extraction can increase Y_{ext} while decreasing w_{marker}. Conversely, a process may improve apparent purity while discarding much of the marker and thereby decreasing R_{marker}. None of these quantities establishes efficacy on its own because the selected marker may indicate identity or stability rather than represent the active constituent.

Comparative bioassays should report results at equal starting-biomass equivalent, equal dry-extract mass and equal quantified-marker concentration. The first comparison examines whole-process productivity, the second describes the crude product as sold, and the third tests whether a particular constituent accounts for the response. Differences in activity at matched marker input leave other constituents, physical state, degradation products or interactions as possible explanations. The rosemary comparison of ultrasound and microwave methods provides evidence of process-dependent selectivity, but its antioxidant-extraction results do not establish an aquaculture treatment effect. [27]

5.6. Concentration, Drying and Intensification Trade-offs

Concentration and drying do more than reduce product volume; both operations can alter its chemical composition. As solvent is removed, the composition, saturation state and viscosity of the liquid phase change. A constituent that is soluble in aqueous ethanol, for example, may precipitate as the ethanol fraction decreases. Operating under reduced pressure lowers the boiling temperature but does not remove the need to monitor residence time, oxygen ingress and entrainment. A useful material balance includes the concentrate, separated solids and, where volatile markers may be lost, the condensate. The concentration factor by itself gives no measure of marker recovery.

Spray drying introduces a carrier or particle-forming stage and exposes the material to a brief heat history that may still be chemically consequential. Freeze drying limits some thermal exposure, but it requires additional equipment and energy and cannot ensure retention of all volatile constituents. Com-parisons between dryers are therefore more informative when they report product temperature, residual moisture, particle structure, redispersibility and marker retention rather than re-lying on the process name. Pilot-scale rosemary research that combined extraction with particle formation also shows that an environmental assessment cannot reasonably stop at the yield of liquid extract; the drying stage must be included. Those operating conditions were specific to the rosemary matrix and should not be treated as a general prescription for heat-sensitive aquaculture phytochemicals. [28]

A comparison of ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) is meaningful only when the chemical targets are matched. Ultrasound may alter tissue accessibility and external mass transfer, although the acoustic energy delivered and the extent of bulk heating depend on the apparatus. Microwave heating is similarly influenced by dielec-tric properties, moisture distribution and equipment geometry; a shorter treatment can still create

localized overheating. In the primary rosemary study, selectivity differed among the ultrasound and microwave configurations. [27] Nominal power and extraction time are consequently inadequate criteria for selecting a scale-up route. Temperature history, recovered marker mass and energy use per accepted product are also needed.

Pressurized hot-water extraction and supercritical carbon dioxide offer further processing options, but they do not recover the same range of compounds. Carbon dioxide may need a modifier when polar constituents are targeted, and its process balance must account for compression, depressurization and co-solvent removal. Hot-water treatment can combine extraction with chemical transformation. Enzyme-assisted disruption of the plant matrix raises separate questions concerning enzyme manufacture, inactivation and carryover, whereas deep eutectic solvent formulations require composition-specific evaluation of toxicity and downstream recovery. A comparative study of rosemary essential-oil extraction found that the higher yield achieved with deep eutectic solvent assistance was not associated with the lowest life-cycle burden. [28, 29] No extraction technology can therefore be classified as universally green with-out reference to its target compounds, operating conditions and full process boundary.

Table 1. Integrated chemical, process, exposure and translation checkpoints. The entries consolidate the detailed distinctions used throughout the review.

Domain	What changes	Consequence for interpretation	Minimum evidence required
Chemical identity	Phenolics, alkaloids, terpenoids, saponins, sulfur compounds and polysaccharides differ in ionization, size, volatility and reactivity. [6, 7]	Total phenolics or a botanical name cannot identify active species or predict one solvent optimum.	Authentication, moisture basis, fingerprint, multiple markers and impurity limits.
Extraction	Solvent, pH, temperature, time, particle size ratio and mixing alter release, co-extraction and degradation. [12, 27]	Higher crude yield may dilute markers or increase transformed material; UAE/MAE do not remove separation duties.	Dry-biomass basis, marker recovery, temperature history, filtrability and material balance.
Finishing and stability	Concentration, drying, oxygen, moisture and storage can cause precipitation, volatilization, oxidation or transformation. [8, 26]	Extraction-stage chemistry may not represent the administered product.	Stability-indicating analysis, recovery after reconstitution, packaging and retained-activity criteria.
Formulation and exposure	Feed binding, leaching, carriers, water exchange, sorption, uptake and metabolism separate nominal dose from exposure. [8, 15]	Equal extract additions can produce unequal freely available or target-site concentrations.	Marker per finished feed, actual intake, concentration–time profile, carrier controls and tissue/water recovery.
Biological design	Direct assays, feeding, injection, immersion and challenge studies address different causal questions.	Broth inhibition, biomarkers and survival cannot be pooled or treated as equivalent efficacy.	Independent tanks/units, relevant comparator, blinding where feasible, attrition and adverse
Bias and reporting	Randomization, blinding, chemical definition, exposure measurement and complete endpoint reporting are often incompletely verifiable.	Apparent benefit may reflect pseudoreplication, selective outcomes or an undefined preparation.	Domain-based appraisal; retain positive, null and adverse findings; mark unavailable information as
Scale-up and economics	Heat/mass transfer, filtration, solvent recovery, drying, quality control and rejected batches change with scale. [28, 29]	Laboratory extraction time or cost per crude kilogram can misrepresent accepted-product performance.	Multiple compliant pilot batches; full cycle time; utilities; cost per specified product.
Sustainability and gaps	Cultivation, solvent manufacture, energy, carriers, farm delivery, discharge and toxicity can shift burdens. [5, 30]	Natural origin, water extraction and biodegradability do not establish whole-system benefit.	Benefit-linked functional unit, uncertainty analysis, nontarget safety and explicit system boundaries.

Table 1 brings together the principal questions concerning extraction, finishing and scale-up.

The preferred process is one that produces a stable, specified preparation with an acceptable resource demand, rather than simply the largest gravimetric yield.

6. Medicinal Plants and Aquaculture Applications

Table 2 records the preparation, dose, route, outcome and main limitation of interpretation for each mapped study. The subsections below compare types of application rather than catalogue plant species. Reported concentrations are experimental conditions, not treatment recommendations.

Table 2. Study-level process–composition–activity evidence map. D: direct assay; H: host experiment; NV: not verified from the accessible source. Doses are experimental observations, not farm recommendations.

Study and preparation	Target, route and dose	Principal finding	Interpretive limit
E01 Andrographis leaf; aqueous/ethanol/methanol extracts [12]	S. agalactiae; in vitro and tilapia feed; aqueous MIC 31.25 μg/mL	Aqueous preparation had the lowest MIC and reduced challenge mortality.	D+H; active chemistry and oral marker exposure unresolved.
E02 Ethanolic Persicaria odorata leaf; catechin/quercetin detected by HPLC [24]	Labeo chrysophekadion; 0.50/0.75 g kg−1 diet; 150 d plus challenge	Challenge survival improved; growth did not improve significantly.	D+H; markers do not establish causal constituents.
E03 GF-7 four-herb mixture; HPLC-DAD	A. hydrophila; 0.1–0.6% diet; 28 d plus challenge	Direct inhibition and host protection were reported.	D+H; mixture-level efficacy, not component attribution.
E04 Olive-leaf extract and oleuropein comparator [31]	Viral haemorrhagic septicaemia virus; cell culture	Infectivity and viral accumulation declined; fusion assay supported an envelope-related	D; no production trial or whole-extract equivalence.
E05 Indian plant/solvent screen [32]	White spot syndrome virus; challenge screen; Cynodon aqueous extract 100 mg kg−1 body mass	Five of 20 plants showed activity with solvent-dependent strength.	H; administration details and broad generalization remain limited.
E06 Oral Cynodon dactylon preparation [33]	Penaeus monodon; white spot challenge; dose NV	Oral protection was reported.	H; title does not establish replicated multi-farm validation.
E07 Nonpolar fractions of Aucklandia, Cnidium and Magnolia [13]	Saprolegnia/Achlya; Aucklandia EC50 11.3/26.1 mg L−1	Petroleum-ether fractions were most active; sensitivity differed 2.31-	D; no hatchery safety window.
E08 Aqueous Terminalia catappa leaf [34]	Tilapia pathogens and egg-associated infection; dose NV	Antiparasitic, antibacterial and egg-protection applications were reported.	D/H boundary; chemical attribution unresolved
E09 Four-solvent Santalum album extract comparison [35]	Goldfish monogeneans; 40 mg L−1 chloroform-derived fraction	Chloroform fraction was most effective; parasites differed in sensitivity.	H; test-specific safety and residual-solvent control.
E10 Garlic extract pellets;	Barramundi/Neobenedenia; feed for 10 or allicin leaching measured [15] 30 d	Infection was up to 70% lower after 30 d, not 10 d; allicin loss was < 3% in the tested interval.	H; product- and feeding-history-specific.
E11 Bioassay-guided Macleaya fractionation; sanguinarine identified by NMR/MS [14]	Grass carp/Ichthyophthirius; 0.9 mg L−1 bath, 48 h	Parasite counts were 96.8% lower; no treated-fish mortality during the trial.	D+H; short safety duration.
E12 Water-extracted Ocimum leaf in feed [36]	Rohu/A. hydrophila; 0.05–1% diet, 42 d	Immune responses changed; relative percent survival reached 40% at 0.2%.	H; marker exposure unresolved.

E13 Tinospora solvent extracts [17]	Tilapia/A. hydrophila; injection, 0.8–80 mg kg−1 body mass	Selected doses altered host responses and protection.	H; injection cannot be converted to feed inclusion.
E14 Hot-water Toona extract [16]	Tilapia/A. hydrophila; injection, 4/8 μg g−1	Innate responses and 7-d survival improved at 8 μg g−1	H; short injected exposure.
E15 Astragalus polysaccharide preparation [23]	Nile tilapia; 1500 mg kg−1 diet	Growth and several immune/antioxidant endpoints changed; NO and MDA did not.	H; molecular distribution and challenge benefit unresolved.
E16 Quillaja saponin mixture [18]	Carp; 150/300 mg kg−1 feed, 8 weeks	Weight gain was 372% at 150 mg kg−1 versus 327% control and 325% at 300 mg kg−1 .	H; small study and nonmonotonic response.
E17 Commercial Artemisia essential oil; vacuum-coated feed [10]	Gilthead seabream; 0.25/0.50% diet, 2 months	Weight gain did not improve; SGR declined, and higher-dose FCR was	H; null/adverse results retained.
E18 Moringa seed extract [11]	Carp water exposure; 96-h LC50 124 mg L−1	Sublethal exposure altered haematological and biochemical endpoints.	H toxicity; seed hazard cannot be generalized to leaf meal.
E19 Commercial G115 ginseng extract [37]	Nile tilapia; 50–250 mg kg−1 diet, 17 weeks	Growth, feed use and blood indices improved versus control.	H; product-specific, not arbitrary ginseng powder.
E20 Ethanolic Cynodon diet [38]	Catla catla; 0.05–5% diet, 45 d	Growth, feed-use and antiprotease responses were reported.	H; overlapping publication records require reconciliation.
E21 Curry/marigold leaf extracts [39]	Rohu/Saprolegnia; 60-d feeding plus 14-d challenge	Combined extracts produced stronger host responses and protection.	H; synergy and field transfer were not established.

6.1. Growth Promotion and Feed Utilization

Botanical feeding studies have tested powders, solvent extracts and proprietary mixtures. Proposed explanations for altered performance include changes in palatability, digestion, intestinal condition and resistance to low-grade disease, but these processes are rarely interchangeable. Ginger has been evaluated in rainbow trout and Asian seabass, while peppermint and garlic studies combined production and health endpoints. Interpre-tation depends on the host species, basal ration, preparation method and feeding period. [40–43]

The studies summarized in Table 2 include ginseng, *Quillaja*, papaya, garlic and *Cynodon*, without implying a shared active substance or transferable inclusion level. Leaf meals introduce an additional complication because they may alter dietary protein, fiber and micronutrient composition; nutritionally matched controls are therefore essential. [18,37,38,44,45] In the *Quillaja* experiment, weight gain reached 372% of starting mass at 150 mg/kg feed, compared with 327% in the control and 325% at 300 mg/kg. The 372% value is not a 372% advantage over the control, and the dose series was not monotonically beneficial. [18] Katuk-leaf results likewise need to be interpreted in relation to inclusion, consumption and water conditions. [46]

Materials such as *Aloe vera*, *Moringa oleifera*, *Ocimum basilicum* and mixed products broaden the range of candidates, but they also reveal problems created by undefined formulations and inconsistent dose denominators. [47] Null and adverse findings help define those limits. Dietary *Artemisia arborescens* essential oil at 0.25% or 0.5% for two months did not improve weight gain in gilthead seabream; specific growth rate declined, and the higher inclusion impaired feed conversion. [10] At the tested inclusions, *Persicaria odorata* improved survival after challenge without producing a significant growth benefit. [24] Disease resistance and production performance are therefore separate outcomes.

An interpretable feeding study reports extract mass and post-processing marker content per unit of feed, then relates these values to intake, digestibility, survival, tissue condition and feed conversion. Whole plant material can alter nutrition as well as chemical exposure. An extract, by comparison, may affect appetite or digestive conditions without supplying

equivalent nutrients. A "growth-promoting" claim requires a measured production benefit accompanied by acceptable health, rather than improvement in an isolated biomarker.

6.2. Antioxidant Chemistry and Immune Modulation

6.2.1. Radical Trapping and Antioxidant Kinetics

Direct radical interception differs chemically from induction or preservation of the animal's own antioxidant defences. Pheno-lic hydroxyl groups may transfer hydrogen atoms or electrons, while conjugation can stabilize the resulting phenoxyl radical. Reaction rate and biological relevance still depend on molecular structure, medium, pH and cellular compartment. A total-phenolic value cannot predict reaction kinetics in fish tissue. [20, 48]

Equation (4) gives a simplified representation of chain-breaking radical trapping:

$$LOO^{\bullet} + ArOH \longrightarrow LOOH + ArO^{\bullet},\ v_{trap} = k_{trap}\,[LOO^{\bullet}]\,[ArOH]. \quad (4)$$

In this conditional model, LOO• denotes a lipid peroxyl radical, ArOH a phenolic donor, and $k$trap a second-order constant for the represented step. The expression is not an empirical rate law for a crude mixture; it omits regeneration, product-radical chemistry and competing substrates. Protection requires trapping to compete successfully with propagation at the site of oxidation. Strong reactivity in an alcoholic assay may not carry over to a membrane when partitioning, metabolism or accessible concentration is unfavourable. Conversely, lipophilicity may concentrate a compound near lipid substrates. Metal binding can restrict catalytic iron or copper, although the redox behaviour of the resulting complex determines whether net protection occurs. [20, 48]

DPPH, ABTS and ferric-reducing methods measure different chemical events. Their IC_{50} values are protocol-dependent and are not kinetic constants. The *P. odorata* report, for example, used a 30-minute DPPH endpoint. [24] Time-resolved assays should specify the radical, medium, temperature, concentrations and blank correction, and should exclude interference from extract color, turbidity or direct reduction of the indicator.

6.2.2. Host Defenses and Biochemical Pathways

Lysozyme, complement, phagocytosis, respiratory burst and antioxidant enzymes represent distinct host functions. Studies of *Ocimum sanctum* and *Toona sinensis* included immune or challenge responses, whereas the *Astragalus* polysaccharide study reported growth, digestive and immune variables without establishing a challenge outcome. [16,23,36] The phagocyte respiratory burst is a regulated antimicrobial response and should not be equated with uncontrolled oxidative damage. Serum measurements, moreover, cannot represent all responses at the skin, gill and intestinal surfaces. [4]

GF-7 combined fractions of Galla chinensis, mangosteen shell, pomegranate peel and Scutellaria baicalensis. Against the tested bacteria, MIC values ranged from 0.045 to 0.36 mg mL−1. Dietary inclusion at 0.1%, 0.3% or 0.6% for 28 days was associated with antioxidant and immune changes, lower malondialdehyde and protection against Aeromonas hydrophila; cytokine- and MyD88-related transcripts were also measured. These results characterize responses to the mixture but do not demonstrate direct binding of each ingredient to a signaling protein. [25]

Antioxidant regulation involving Nrf2 and inflammatory signaling involving NF-κB offer plausible interpretive pathways, but altered transcript abundance alone does not establish pathway activity or molecular engagement. Stronger evidence would include chemical exposure, protein activity or localization, perturbation tests and tissue-specific outcomes. [4, 47] Route of administration also changes the question being tested. Water-extracted *Ocimum* was fed before challenge, whereas *Tinospora* and hot-water *Toona* preparations were injected. [16, 17, 36] Some *Astragalus* endpoints, including nitric oxide and malondi- aldehyde, remained unchanged. [23] Evidence for direct radical trapping is not equivalent to evidence for altered host expression, and oral and injection studies face different exposure barriers.

6.3. Antibacterial Activity and Chemical Interactions

Studies involving Aeromonas, Vibrio, Streptococcus and related pathogens commonly report one of two non-equivalent outcomes: inhibition in microbial culture or reduced disease susceptibility after administration to the host. An oral extract may alter host immunity without reaching a bactericidal concentration in tissue. Conversely, inhibition in broth establishes neither oral availability nor safety in an aquaculture system. [49] Some lipophilic oil constituents may partition into bacterial membranes and disturb packing, permeability or ionic gradients. Differences in hydrophobic surface and phenolic substitution provide plausible explanations for unequal activity, but they do not support one membrane mechanism for every extract. [50] Garlic thiosulfinates can react with accessible thiols. Tannins and related polyphenols associate with proteins through hydrogen bonding, hydrophobic interactions and, after oxidation, possible covalent chemistry. Protein-rich feed or assay medium may therefore consume, bind or redistribute active material before it reaches the pathogen. [4, 20]

Reduced biomass during growth inhibition is insufficient evidence for effects on quorum sensing, adhesion or biofilm formation. Subinhibitory tests, growth-matched controls, signal reporters or direct signal analysis, together with viable counts, can separate signaling effects from general toxicity. In the same

way, reduced macromolecular synthesis following membrane failure does not establish direct inhibition of a polymerase or ribosome. [7, 49]

The aqueous *Andrographis paniculata* preparation had a reported MIC of 31.25 μg/mL against *Streptococcus agalactiae*. Water, ethanol and methanol extracts were not equally active, and feeding experiments indicated preparation-specific protection in Nile tilapia. These results neither identify andrographolide as the causal compound nor convert the MIC into an immersion dose. [12] Host studies have also examined *Achyranthes aspera*, *Withania somnifera*, *Solanum trilobatum* and peppermint. [51–54] Direct inhibition by tested *Ceramium*, *Laminaria* and *Asparagopsis* preparations does not establish their effectiveness as oral medication. [55, 56]

A claim of synergy requires component controls, fixed ratios and an explicit additivity model. The same requirement applies when extracts are combined with probiotics, vaccines or conventional agents. Gram status alone cannot predict susceptibility to a complex preparation because solvent selection, medium binding, inoculum, contact time and degradation can alter apparent potency. Without measurements of tissue exposure, protection in a challenge experiment cannot show that the host attained a directly antibacterial concentration. [12, 25]

6.4. Antiviral Applications

Olive leaf, pomegranate and several Asian medicinal plants have been tested against viruses of fish or shrimp. For the viral hemorrhagic septicemia virus, both olive-leaf extract and oleuropein were examined in vitro, allowing a mixture and a named constituent to be considered within the same line of evidence. [31] Studies of *Cynodon dactylon* and other Indian plants against white spot syndrome virus have included oral administration, although interpretation remains conditional on the infection model and delivery procedure. [32, 33]

Oleuropein is a glycosylated secoiridoid phenolic, whereas other proposed antiviral agents include flavonoids, alkaloids and terpenoid-related molecules. Outcome data alone cannot distinguish effects on viral entry or replication from host modulation. Time-of-addition designs, viral titration and host-cell viability measurements are needed because nonspecific cytotoxicity can also reduce the viral signal. [57] Findings for *Phyllanthus*, *Clinacanthus nutans* and multi-herb products support further testing, not farm-scale generalization. Viruses differ in envelope, genome, entry and host dependence; antibiotics have no direct antiviral action. [19, 57]

In the Indian screen, aqueous *Cynodon dactylon* and methanolic *Momordica charantia* performed more strongly than the selected *Aegle marmelos*, *Lantana camara* and *Phyllanthus amarus* extracts, which produced partial effects. Protection was not uniform across the five plants. [32] A separate oral *Cynodon* study examined delivery under a different design, but its title alone does not establish replicated validation across farms. [33] Fusion assays provide more specific support for an envelope-related hypothesis for the olive preparation than mortality data alone, although selectivity controls remain necessary for both the whole extract and isolated oleuropein. [31] Virus preincubation, prophylactic feeding and treatment of established infection address different experimental questions.

6.5. Antifungal and Anti-Oomycete Applications

Terminalia catappa and Chinese medicinal plant extracts have been investigated for external infections, but the target organisms require precise classification. *Saprolegnia*, *Achlya* and *Aphanomyces* are oomycetes rather than true fungi. Activity against them is anti-oomycete and cannot automatically be extended to a fungal target. [13, 34] Membrane or protein inter- Actions may contribute to the activity of particular preparations, but an ergosterol-based mechanism derived from a fungal system is not transferable to organisms with different envelope chemistry. Results against *Candida* or *Aspergillus*, together with the diverse mangrove evidence, remain discovery findings until tested in the relevant aquatic infection. [58]

In the Chinese study, petroleum-ether fractions of *Aucklandia lappa*, *Cnidium monnieri* and *Magnolia oflcinalis* were more active than the comparison fractions. The *A. lappa* fraction produced mycelial EC_{50} values of 11.3 mg/L against *Saprolegnia* and 26.1 mg/L against *Achlya*. These values apply to a particular fraction and endpoint, not to the higher concentration used in the initial screen. [13] Feeding curry/marigold leaves before *Saprolegnia* challenge tested host protection; it did not demonstrate sterilization of culture water. [39]

Hatchery evaluation should measure pathogen viability alongside embryo survival, hatch, larval quality and water chemistry. Adsorption to eggs, reactions with dissolved organic matter, and changes in pH can separate nominal from effective concentration. Activity in a nonpolar fraction indicates solvent enrichment but does not identify the active molecule; solvent removal and redispersion may further alter exposure. Practical use depends on identifying a treatment window that is acceptable for the pathogen, host and culture water together.

6.6. Antiparasitic Applications

Preparations tested against monogeneans and ciliates include *Santalum album*, dietary garlic and *Macleaya cordata*. *San-talum* extracts were evaluated against monogeneans in goldfish, whereas garlic was tested against *Neobenedenia* in

bar-ramundi. [15, 35] Bioassay-guided investigation of *Macleaya* linked the identified alkaloid sanguinarine to an *Ichthyophthirius multifiliis* challenge in grass carp, providing stronger chemical attribution than a qualitative phytochemical screen. [14]

Essential oils, ginger and garlic provide additional candidates, but parasite-specific pathways remain uncertain. Membrane perturbation, enzyme effects and host modulation are proposed explanations until demonstrated in the target organism. [50] The parasite stage also affects interpretation. Activity against exposed motile forms does not establish efficacy against protected eggs or cysts, and a prophylactic diet is not equivalent to a short bath. Garlic-fed barramundi showed reduced *Neobenedenia* infection after 30 days but not after 10, indicating that the observed response depended on exposure duration. [15] In the *San-talum* comparison, four solvents yielded preparations to which *Dactylogyrus intermedius* and *Gyrodactylus elegans* differed in sensitivity. [35] NMR and mass spectrometry supported the identification of sanguinarine in the *Macleaya* study. [14] These studies address sustained host exposure, solvent-selective recovery and compound identification, respectively; they do not establish a common mechanism.

Future tests should assess parasite burden together with survival, behavior, gill integrity and post-treatment recurrence. Carrier controls and stage-specific assays are needed to distinguish durable control from temporary immobilization or detachment. Activity in a chloroform-derived fraction does not justify adding chloroform to culture water, and the dose of an isolated alkaloid cannot be transferred directly to leaf powder.

7. Chemical Stability, Storage and Formulation

7.1. Stability Is a Property of a Preparation in a Matrix

A chemical profile obtained immediately after extraction may not represent the material at administration. Constituents can be generated, lost or transformed during tissue disruption, extraction, concentration, drying, storage and formulation. The loss of a chromatographic peak may reflect reaction, precipitation, or adsorption rather than mineralization or disappearance of all biological activity. Garlic provides a clear example: allicin is formed during preparation and may subsequently degrade, so its manufacturing history is part of the biological interpretation. [26]

Primary stability experiments are more informative than assigning a generic “heat-sensitive” label to every phenolic. Under the conditions studied by Wang and colleagues, allicin in aqueous garlic extract was relatively more stable around pH 5–6 and degraded more rapidly at strongly acidic or alkaline pH and at elevated temperatures. Light did not produce the same effect in that system. [26] Protecting a mixed preparation from light may still be prudent, but photodegradation cannot be assumed for every constituent. Nor can the shelf life of a concentrated stock solution be transferred directly to a dilute immersion bath.

Curcumin separates chemical degradation from physical instability. In an experimental emulsion study, pure curcumin degraded in alkaline aqueous media but tended to crystallize in acidic aqueous solutions. Oil-in-water delivery improved dispersibility and retention under the conditions tested, although the experiments did not involve feeding fish. [8] Separate chemical work demonstrated autoxidative cyclization and oxygen incorporation. Disappearance from buffer should therefore not be described simply as hydrolysis to vanillin and ferulic acid. [59] Residual parent compound and transformation products may differ in activity and toxicity, making it necessary to measure the chemical species present during aquaculture exposure.

Stability assessment requires prespecified chemical and functional acceptance criteria, including marker retention, formation of relevant degradation products, physical dispersion or release, microbial quality and retained activity. Accelerated testing can identify vulnerable formulations, but extrapolation is defensible only when the degradation mechanism remains unchanged across temperature or humidity conditions. A measured first-order decline of a parent compound can support a conditional half-life, not a universal half-life for the entire extract. The practical question is whether the stored product still delivers the specified exposure, rather than whether its color appears unchanged.

7.2. Feed Incorporation, Coating and Oral Delivery

Oral administration fits routine feeding practice, but the relevant dose is the amount consumed and retained rather than the mass added to the mixer. Thermal processing, extrusion, drying and storage may alter parent compounds, while pellet ingredients can bind phenolics or change dissolution. Applying a coating after processing reduces some heat exposure but places the material at the pellet surface, where it may leach before ingestion. Formulation comparisons should therefore include marker recovery after manufacture, stability during storage, leaching during water contact and measured feed consumption. [15, 22]

The garlic–barramundi study is informative because it links a prophylactic feeding period with measurements of allicin leaching from the pellets. Low loss during the relevant water-contact interval supports that specific delivery method, but it does not establish equal allicin retention in all pressure-extruded feeds. [15] In the Artemisia experiment, essential oil was incorporated with fish oil by vacuum coating, yet the technically feasible product did not improve growth. [10] The useful formulation question is not simply whether a powder or

coating can be manufactured, but whether it produces the intended exposure and response.

An oral marker balance should express inclusion as mg marker/kg finished feed and combine it with the actual daily feed intake per unit of animal biomass. Even then, ingested dose is not equivalent to systemic exposure because digestive release, transformation, intestinal uptake and first-pass metabolism may intervene. Nominal inclusion should be reported with biomass-specific feeding rate and uneaten-feed losses. An injected dose in mg/kg body mass cannot be recast as a feed inclusion in mg/kg diet, as illustrated by the routes used for Tinospora and Toona. [16, 17]

7.3. Encapsulation, Nanoformulation and Release

Encapsulation may be used to address volatility, poor water dispersibility, oxidation or premature release, but each is a separate formulation objective. A carrier that retains a compound during storage may also restrict its release at the intended site. Emulsion stability does not demonstrate molecular solubility, just as a stable particle-size distribution does not establish bioavailability. The curcumin emulsion model improved physical handling and chemical retention without independently showing greater uptake in fish. [8] Nanoformulation studies in aquaculture identify possible delivery strategies; they do not establish that smaller particles are invariably safer or more effective. [60]

At a minimum, formulation studies should compare free ex-tract, blank carrier and loaded carrier at matched marker input, together with an appropriate conventional preparation. Relevant measurements include encapsulation efficiency, loading per unit carrier mass, marker mass balance, particle or droplet distribution, redispersibility, storage stability and time-resolved release. High encapsulation efficiency can coexist with poor recovery of the total product. Biological testing should determine whether the formulation changes pathogen selectivity, host exposure or carrier toxicity, rather than reporting only a larger in vitro inhibition zone.

Table 1 summarizes the analytical checkpoints needed to connect extract manufacture and stability with feed or immersion studies.

8. From Extract Dose to Aquatic Exposure

8.1. Nominal Dose and Available Fractions

Ten milligrams of extract per liter does not necessarily provide ten milligrams of active compound per liter. If Cext the nominal dry-extract concentration and, nom is pendently measured marker mass fraction, the initial marker concentration is

$$C_{\text{marker,nom}} = C_{\text{ext,nom}} w_{\text{marker}}. \quad (5)$$

This equation is a bookkeeping identity rather than a bioavail-ability model. A hypothetical extract containing 2% marker and added at 10 mg/L would provide a nominal marker concen-tration of 0.2 mg/L before dissolution or loss is considered. The example is neither a measured composition nor a recommended dose for any plant.

After dosing, a marker may be present as freely dissolved molecules, colloid-associated material, particles, sorbed mate-rial or transformation products. Even an operationally dissolved fraction may contain small colloids that pass through the selected filter, while the filter and sampling surfaces may themselves remove solute. "Dissolved," "freely available" and "biologically available" therefore require separate operational definitions. A lipid-based formulation can disperse an oil in water without dissolving every constituent at the molecular level, so chemi-cal assays need to be interpreted alongside measurements of physical stability. [8]

Salinity, pH, suspended matter, dissolved organic material and mixing can all change the distribution of an applied prepa-ration. Exposure assessment should begin by establishing analytical recovery in the actual water matrix and in a blank vessel. Spatial and temporal concentrations should then be mea-sured rather than assuming immediate homogeneity. Marker depletion in a vessel without animals can identify some physical and chemical losses separately from net animal-associated up-take, although microbial transformation and reversible sorption may require further controls. A higher dissolved concentration does not represent improved selectivity if host toxicity increases together with pathogen inhibition.

8.2. Exposure Dynamics and Repeated Dosing

For a well-mixed treatment at constant volume, a screening balance for a defined aqueous marker concentration is

$$\frac{dC_w}{dt} = \frac{\dot{m}_{in}}{V} - \frac{Q}{V} + k_{loss}\, C_w. \quad (6)$$

Here C_w is the measured aqueous marker concentration, $\dot{m}_{in}$ is the marker input rate to the modelled phase, V is water volume, Q is water-exchange flow and k_{loss} is a conditional first-order loss coefficient. For a slowly dissolving preparation, input to the aqueous phase is governed by the release rate rather than simply by the amount of crude extract added.

The balance assumes marker-free replacement water; Q/V and k_{loss} have units of inverse time. With no continuing input and constant coefficients, parent concentration declines exponentially at the rate $Q/V + k_{loss}$. This remains a proposed screening model, not a validated simulation of pond conditions.

The balance identifies several ways in which a nominal bath concentration can change. Greater water exchange shortens residence time, whereas a second dose may accumulate if the first has not dissipated. The concentration–time integral, AUCw = Cw(t) dt, summarizes external marker exposure but does not establish that equal integrals produce equal effects. Peak concentration, time above a relevant threshold and para-site life stage may contribute independently. Dose schedules should be evaluated against measured concentration profiles and biological responses rather than derived only from an in vitro MIC.

Reversible sorption, carrier release, saturable uptake, micro-bial transformation and variable flow require additional com-partments or time-dependent terms. A single disappearance coefficient may fit the observed curve while misrepresenting the underlying processes and performing poorly after scale-up. Studies should report sampling recovery, detection limits, phase definitions, temperature, water exchange and uncertainty in fitted parameters. Loss of the parent compound must also be distinguished from transfer to sediment or organisms; otherwise apparent clearance may conceal continued exposure through another compartment.

8.3. Exposure-Aware Safety and Practical Administration

Immersion exposes host surfaces, pathogens and nontarget or-ganisms at the same time. A therapeutic window must therefore compare compatible routes, matrices and exposure durations. A ratio between an in vitro MIC and an unrelated acute fish-toxicity endpoint is not sufficient. Measured toxicity of *Moringa* seed extract in carp and of essential oils in *Daphnia* establishes the need for selectivity testing, but it does not provide a univer-sal hazard classification for every part or preparation of those plants. [5, 11] Safety assessment must extend beyond survival during a brief challenge to early-life stages, chronic growth, gill integrity, behavior, reproduction and residue depletion.

Closed baths provide greater control over exposure duration and discharge than open ponds or cages, although the concen-trated used water remains an effluent that requires assessment. Compatibility testing in recirculating systems should include nitrification and other beneficial treatment microorganisms. Feed delivery presents a different problem: appetite may de-cline during disease, reducing uptake even when the nominal provision of medicated feed increases. Injection studies can reveal host responses, but they avoid the barriers governing oral uptake and provide no evidence that administration would be inexpensive or scalable. [16, 17]

Decisions about practical use require a defined preparation, a measured exposure profile and safety evidence relevant to the intended route. Feeding trials should quantify both extract and marker mass per kilogram of finished feed, record actual consumption and calculate marker intake relative to animal biomass. Immersion experiments need corresponding measure-ments of concentration over time, water exchange and treatment duration. Such route-specific data support interpretation with-out implying that a universal botanical dose exists. Botanical origin is not a substitute for exposure measurement, and loss of the parent marker does not demonstrate food safety. Residues, metabolites and formulation constituents must be considered as separate analytical questions.

9. Scale-Up, Techno-Economics and Environmental Sustainability

9.1. From Laboratory Extraction to Consistent Production

Scale-up cannot be achieved by increasing solvent and biomass quantities while leaving extraction time unchanged. Equipment size affects heating and cooling, suspension quality, local en-ergy delivery, solvent retained in spent biomass and separation throughput. A credible pilot demonstration should close the ma-terial balance, measure utility use and compare several accepted batches against the same chemical and functional specification.

The corresponding evidence requirements are listed in Table 1. Production control has to account for variation in the starting material. Harvest timing, plant part, moisture and storage his-tory can alter the composition entering the extractor. Changes in the ethanol/water ratio of recycled solvent may then alter the fraction recovered. Incoming-lot acceptance, extraction control and final-product release should therefore be treated as separate control stages. Blending may reduce variation when marker contents and contaminants are known, but it cannot make an uncharacterized extract chemically uniform. Testing must also cover the product after actual feed processing or dilution; an extraction-stage fingerprint alone is insufficient for release.

Recovering solvent can reduce the demand for fresh inputs, although the recycled stream may carry water, volatile con-stituents or contaminants that accumulate over repeated use. Closing the solvent balance requires measurement of fresh makeup, recovered solvent, entrainment losses and purge. The choice between extraction at the production site and centralized manufacture also depends on the transport of wet biomass, solvent safety, operator expertise, utility reliability and access to quality-control facilities. These factors vary among locations, so neither decentralized nor industrial production is preferable in all settings.

9.2. Case-Based Translation Across Preparation Types

Four cases illustrate why practical recommendations need to refer to the tested preparation rather than to the plant name. In the *Andrographis* comparison, the aqueous fraction pro-duced the lowest reported MIC in a defined bacterial

assay, identifying solvent selection as the most immediate process variable. The chemical markers, stability in feed and oral ex-posure were not resolved. [12] In barramundi receiving garlic, reduced monogenean infection was associated with a 30-day feeding period, and leaching from the pellets was measured. These measurements allow formulation and exposure history to be considered together instead of being inferred from garlic inclusion alone. [15]

Bioassay-guided fractionation of *Macleaya cordata*, followed by spectrometric identification of sanguinarine, provides more specific chemical attribution than a crude-screening result. Ev-idence on chronic host safety and practical delivery is still required. [14] The rosemary studies address a different part of development: they compare extraction selectivity, drying and life-cycle burdens at bench or pilot scale but do not establish efficacy in aquaculture. [27–29] Considered together, these cases support a staged sequence in which a preparation-specific advantage is first established, its chemical basis is examined, delivery through the intended route is measured, and pilot-scale cost and environmental burden are then compared.

9.3. Techno-Economic Assessment and Comparable Functional Units

A techno-economic assessment (TEA) should estimate the cost of producing material that meets defined marker, stability and activity specifications. Cost per kilogram of crude extract may favour a high-yield process that mainly recovers inactive solids. At minimum, the costing boundary should include biomass preparation, solvent purchase and recovery, utilities, separation, drying, carriers, labor, analysis, rejected batches, equipment utilization and capital charges. Currency, price year, location, production capacity and financing assumptions must also be re-ported. A common cost benchmark was not calculated because the aquaculture studies do not provide a harmonized process inventory.

A comparative study of four rosemary essential-oil extrac-tion routes provides a nonaquatic example integrating yield, environmental burdens and projected economics. Deep eutectic solvent assistance increased extraction yield, but upstream bur-dens weakened its environmental ranking.

The preferred route depended on the complete modelled process rather than the solvent's green label. [29] This study illustrates a comparative method; its modelled selling price, payback and energy use cannot be transferred to another plant, country or aquaculture product, nor treated as evidence of farm benefit.

For aquafeed applications, product cost must ultimately be related to inclusion level, treatment duration, actual feed intake, survival and feed conversion. In immersion treatments, labor, treatment volume, water exchange, effluent management and number of applications may dominate delivery cost. Avoided antibiotic use, reduced mortality or market premiums belong in the base-case model only when demonstrated against an appropriate comparator. Otherwise, they should appear as clearly labelled sensitivity scenarios rather than asserted commercial advantages.

9.4. Life-Cycle Boundaries and Burden Shifting

Life-cycle assessment (LCA) should distinguish a gate-to-gate extraction comparison from an assessment extending from biomass production to farm use and discharge. Cultivation or collection, drying, solvent manufacture and recovery, carrier production, transport, farm energy and disposal can all change the ranking of alternatives.

Water extraction may still require substantial energy for evaporation or freeze drying. A low-volatility solvent may be difficult to remove and could introduce an additional exposure concern. Comparisons should therefore use chemically and functionally equivalent preparations rather than treating natural feedstock as evidence of low environmental burden.

Pilot-scale rosemary research comparing integrated water extraction and particle formation with separately dried extracts shows that the drying boundary and electricity supply can affect the result. [28] A later essential-oil study also identi-fied electricity and solvent production as important sensitivity variables. [29] Neither study measured downstream aquacul-ture benefits or aquatic ecotoxicity. Their process rankings therefore cannot establish the whole-system sustainability of a fish-health intervention. That assessment would require a validated manufacturing inventory linked to measured farm use and discharge.

Fruit-processing by-products may reduce reliance on purpose-grown biomass. [30] Their use still involves collection, seasonal availability, drying, contamination control and competition with other applications. Avoided-disposal credits require an explicit counterfactual, while conclusions should be tested against alter-native co-product allocation methods. Reported essential-oil effects on *Daphnia* also show that biodegradability and resource efficiency do not establish aquatic safety. [5]

10. Research Gaps and Future Directions

10.1. The Missing Links Are Often Between Measurements

The available evidence reports nominal doses and biological endpoints more often than it links raw-material characteristics with recovery, storage and exposure. The field does not lack candidate plants; it lacks comparable preparations followed from production through administration. Table 1 identifies the measurements required to address these gaps.

Chemical standardization cannot rely on a single total-phenolic measurement, although preliminary evaluation does not require identification of every constituent. A workable tiered specification could include authentication, a reproducible chro-matographic fingerprint, several quantitative markers, impurity limits and evidence that activity is retained.

When fractionation yields a candidate active principle, recombination experiments can test whether the crude extract matrix alters activity, antagonism, stability or delivery. The identification of sanguinarine provides more direct chemical attribution than a crude phyto-chemical screen; however, the brief efficacy experiment leaves chronic safety and ecosystem effects unresolved. [14]

Biological trials must use the actual experimental unit. Fish within a tank share diet and water, so treating each fish as an independent replicate can overstate precision when treatment is assigned to the tank. Development studies should use ran-domized replicate tanks or independently assigned production units, prespecified primary outcomes, justified sample sizes, blinded measurements where feasible and complete reporting of withdrawals and mortality.

Basal diets, feeding rates, carrier controls, pathogen identity and challenge route also need to be comparable before observed differences are attributed to extraction chemistry. When many biomarkers are measured, uncertainty and multiplicity should be addressed rather than selecting only favourable endpoints.

Dose optimization must include adverse and null responses as well as the apparent optimum. The *Quillaja* and *Artemisia* examples show that increasing supplementation does not neces-sarily increase growth and that selected immune changes do not compensate for an unfavourable production outcome. [10, 18] A useful experiment defines a dose window for a specified preparation and route while measuring exposure and adverse effects. A single favourable inclusion should not be extrapolated across animal sizes, species or culture systems.

Process-development experiments should link each step: vary a process condition, measure the resulting composition, verify stability and delivery, and compare biological responses on interpretable dose bases. An association between marker abundance and efficacy can generate a hypothesis, but it does not establish molecular causation when several constituents change together.

Independently produced process batches, targeted enrichment or depletion and chemically matched controls can help distinguish processing effects from biological variation. Scale-up should then determine whether the same specification remains achievable without unacceptable increases in solvent consumption, energy use, waste generation or cost.

11. Limitations of the Evidence Base

This narrative review was not based on exhaustive database coverage, duplicate screening or literature retrieval in every language. The searches instead focused on verifying individual claims and integrating evidence across themes. Inaccessible full texts and selective publication of favorable outcomes may therefore influence the apparent balance of evidence.

The structured appraisal identifies recurring concerns in design and reporting, but it cannot reconstruct unreported randomization, blinding, attrition or analytical recovery. These constraints limit certainty and prevent estimates of how frequently particular findings or weaknesses occur across the wider literature.

The source studies differ in preparation chemistry, dose de-nominator, species, route, duration and endpoint, so they do not support a universal dose, extraction constant or pooled effect size. This limits direct prescribing, but a combined estimate would introduce misleading precision. Route-specific marker balances, the concentration–time model and within-study con-trasts instead identify the measurements needed to develop quantitative guidance for a defined preparation. Any such guid-ance remains conditional until consistency across independent batches, exposure and safety have been demonstrated.

12. A Stage-Gated Model for Future Research

The development model in Figure 3 requires evidence at each transition. A positive assay alone does not justify progression to commercial use.

The identity gate requires a repeatable source and extraction process. At the chemical gate, each marker should have a stated purpose, such as confirming identity, tracking an activity asso-ciation or monitoring degradation, rather than being selected only because an analytical standard is convenient. The delivery gate asks whether stored and formulated material retains its specification and produces measurable exposure at the intended site. Safety evaluation then addresses host and nontarget toler-ability under realistic concentrations, durations and discharge conditions. Toxicity screening should begin earlier and continue throughout development; the sequence is a decision framework, not a reason to postpone safety testing.

The final gate combines aquaculture benefit with process feasibility. Pilot batches should reproduce the preparation used in the efficacy trial, while TEA and LCA should compare alternatives using an explicit functional unit and uncertainty analysis. If the formulation or solvent changes after validation, chemical comparability must be reassessed and exposure or effi-cacy testing repeated where necessary. Failed batches, adverse outcomes and ineffective doses remain part of the evidence base.

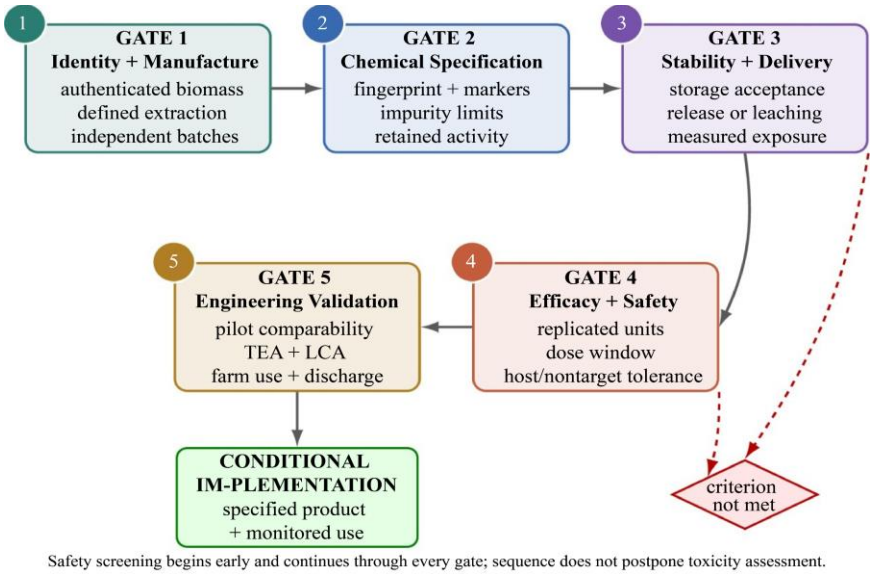


Fig. 3 Proposed stage-gated development model for medicinal plant preparations in aquaculture. Evidence checks connect identity and manufacture, chemical and functional definition, delivery and safety, and aquaculture and engineering validation. Progression to commercial implementation is conditional on demonstrated specifications, benefit and safety. TEA: techno-economic assessment; LCA: life-cycle assessment.

The relevant question is not simply whether a plant extract works, but which chemically defined preparation works under specified processing conditions and biologically relevant exposure, with acceptable stability, safety and manufacturing performance. A report of improved survival or growth in one study does not establish that the preparation has passed the other development stages.

12.1. Operational Visual Synthesis

Figures 4 and 5 separate two parts of the development sequence. Figure 4 follows material and solvent streams through batch release, whereas Figure 5 traces the divergence between nominal addition, available fractions and target-site exposure.

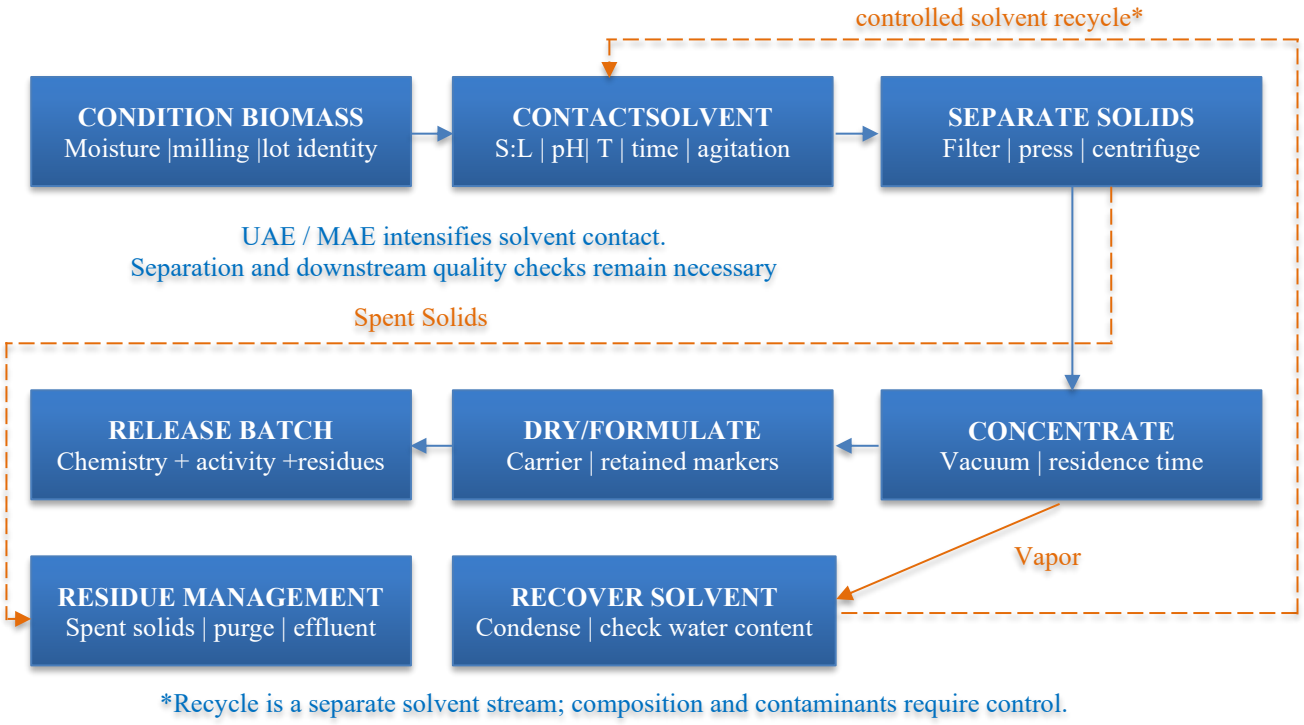


Fig. 4 Extraction and downstream-processing workflow for a standardized aquaculture preparation. Contact intensification by UAE or MAE does not replace solid–liquid separation, solvent control, marker recovery, formulation and batch-release testing.

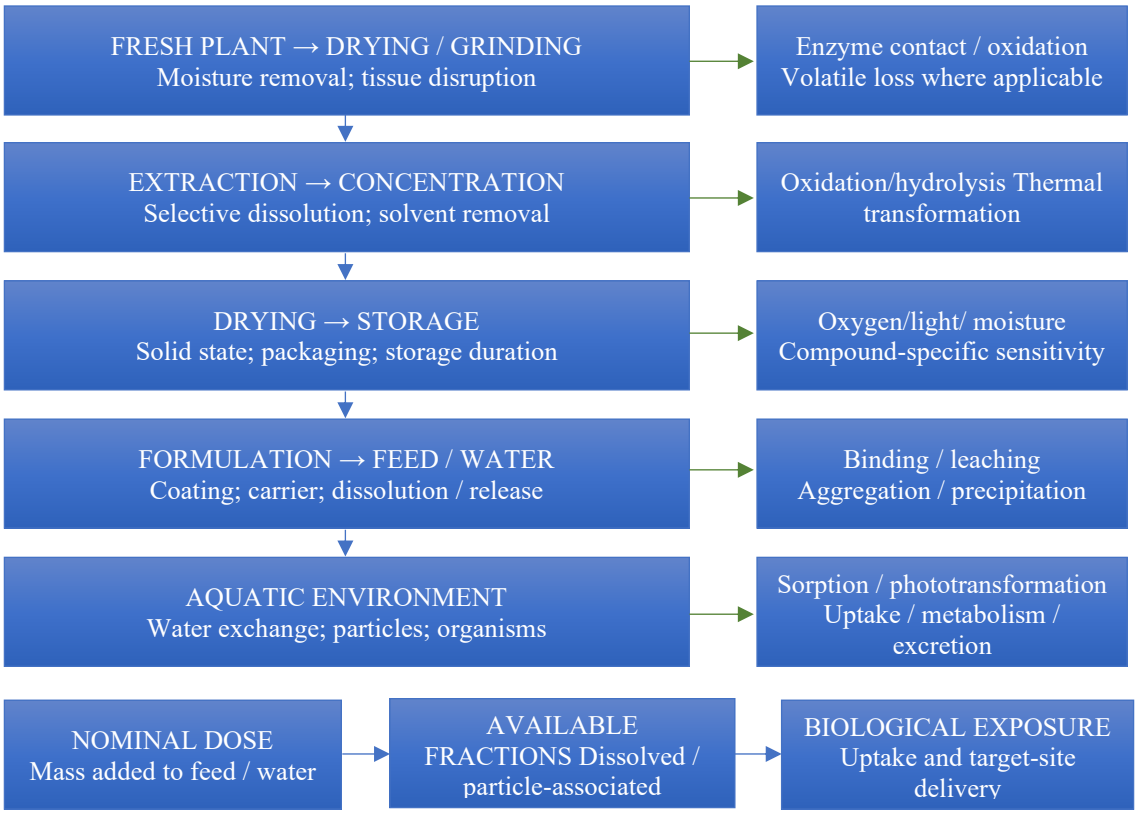


Fig. 5 Conditional chemical fate from plant preparation to aquatic exposure. Physical removal, chemical transformation and biological uptake are distinct processes; nominal dose, available fractions and target-site exposure are not interchangeable quantities. [5, 8, 15, 26, 59]

13. Conclusion

A medicinal plant cannot be defined as an aquaculture intervention by its botanical name alone. The composition of the material introduced into feed or water reflects the history of the biomass and the methods used for extraction and finishing. Storage, formulation and administration route then affect which constituents can reach the host or pathogen. The coexistence of favorable, neutral and adverse findings in the reviewed literature supports evaluating each preparation on its own terms. Natural origin provides no assurance of safety or low environmental burden.

Manufacture and biological response are closely connected. Molecular structure influences solvent affinity, membrane partitioning, redox behaviour and protein interactions, whereas processing determines recovery, transformation and release. Once the preparation has been administered, dilution, sorption, degradation and uptake can widen the difference between the nominal dose and exposure at the target site. Radical trapping, microbial inhibition, immune regulation and other host responses may contribute to the same endpoint, but they require different evidence.

Further development requires authenticated biomass, controlled manufacture, fit-for-purpose chemical specifications, stability and delivery measurements, route-specific safety testing, and replicated aquaculture trials. Pilot lots must remain comparable with the preparation used to establish efficacy.

Economic and life-cycle assessments should include recovery operations, energy, carriers, rejected production, farm administration, and discharge rather than relying on crude-extract yield. Commercial consideration is justified only when a defined process produces reproducible chemistry, beneficial exposure and acceptable biological, environmental and economic performance.

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Declarations

Author Contributions

Arnab Chatterjee: Conceptualisation, writing—original draft, and writing—review and editing. Su-tapa Sanyal: Supervision, guidance, and critical review of the manuscript. Dipan Adhikari: Supervisory support and review of the manuscript.

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Consent to Participate

Not applicable.

Consent for Publication

Not applicable; no identifiable participant data are reported.

Data Availability

No new experimental datasets were generated or analysed. The evidence discussed in this review is derived from the publications cited in the reference list.

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