

Bioactive Compound Isolation from Medicinal Plants

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ABSTRACT

Bioactive compound isolation from medicinal plants is the foundational methodology underpinning modern phytopharmaceutical development and the translational understanding of classical Ayurvedic and other traditional-medicine therapeutics. The methodological pipeline typically spans raw material selection and authentication, extraction with appropriate solvent and technique selection, chromatographic separation and purification, spectroscopic structure elucidation, and activity characterisation. Over the past decade substantial methodological advances have reshaped each stage: green-chemistry extraction techniques including supercritical CO₂ extraction and subcritical water extraction have supplemented or replaced traditional Soxhlet methodology; high-throughput chromatographic methodology and preparative HPLC have accelerated purification; high-field NMR and high-resolution mass spectrometry have refined structure elucidation; bioassay-guided fractionation approaches have systematised the connection between isolated compounds and therapeutic activity. This study reviews 52 primary phytochemistry studies published between 2014 and 2022 on bioactive compound isolation from Ayurvedic and related medicinal plants, grouped under five methodology frames: BI1 (extraction methodology development and comparison), BI2 (chromatographic separation and purification methodology), BI3 (spectroscopic structure elucidation methodology), BI4 (bioassay-guided fractionation approaches), and BI5 (scale-up and standardisation for pharmaceutical deployment). Each frame was scored with the Bioactive Compound Isolation Methodology Index (BCIMI). Bioassay-guided fractionation (BI4) scored highest at BCIMI 0.816, with spectroscopic structure elucidation (BI3) at 0.798 and chromatographic separation (BI2) at 0.780.

Keywords: bioactive compound isolation; medicinal plants; Ayurvedic phytochemistry; supercritical CO₂ extraction; preparative HPLC; NMR structure elucidation; bioassay-guided fractionation; LC-MS; green chemistry extraction; phytopharmaceutical scale-up; BCIMI

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1. Introduction

1.1 Bioactive Compound Isolation as Foundational Phytochemistry

Bioactive compound isolation from medicinal plants is a foundational methodology in modern phytopharmaceutical development. The methodological pipeline connects raw plant material, through systematic extraction and purification, to isolated bioactive compounds with characterised chemical structure and biological activity. For Ayurvedic and related traditional-medicine contexts, the pipeline supports translational understanding by identifying specific bioactive compounds responsible for classical therapeutic claims, characterising their pharmacological targets, and enabling modern pharmaceutical development of standardised single-compound or defined-extract preparations. The methodology spans five interconnected stages: raw material selection and authentication; extraction using appropriate solvent and technique selection; chromatographic separation and purification; spectroscopic structure elucidation; and biological activity characterisation. Each stage has mature methodology with ongoing methodological refinement.

1.2 Methodological Advances and Study Objectives

Over the past decade substantial methodological advances have reshaped each stage of the isolation pipeline. Green-chemistry extraction techniques including supercritical CO₂ extraction, subcritical water extraction, ultrasound-assisted extraction, and microwave-assisted extraction have supplemented or replaced traditional Soxhlet methodology with environmental and efficiency advantages. High-throughput chromatographic methodology and preparative HPLC have accelerated purification workflows. High-field NMR (600-900 MHz) and high-resolution mass spectrometry including Orbitrap and Q-TOF instruments have refined structure elucidation capabilities. Bioassay-guided fractionation approaches have systematised the connection between isolated compounds and therapeutic activity, moving beyond classical-literature-based compound selection to activity-driven compound identification. This review organises the published methodology evidence along five frames. The paper follows the standard six-part structure.

2. Literature Review

2.1 Extraction and Chromatographic Separation Methodology

Extraction methodology development and comparison (BI1) has been a particularly active area. Classical Soxhlet extraction with sequential solvent polarity remains the baseline methodology but has limitations including extended extraction time, thermal degradation risk, and solvent waste. Supercritical CO₂ extraction has documented advantages including reduced thermal exposure, tunable selectivity through pressure and temperature adjustment, and minimal solvent residue (Sharma and Kumar, 2015; Reverchon and De Marco, 2017). Subcritical water extraction has particular value for polar bioactives traditionally extracted by aqueous decoction methodology. Ultrasound-assisted and microwave-assisted extraction techniques reduce extraction time substantially with preserved or enhanced yield. Chromatographic separation and purification methodology (BI2) provides the purification backbone. Silica-gel column chromatography remains widely used but is being supplemented by flash chromatography, HPLC, preparative HPLC, and countercurrent chromatography. UPLC-MS and LC-MS-guided fractionation have accelerated purification workflows (Ventura et al., 2017).

2.2 Spectroscopic Structure Elucidation and Bioassay-Guided

Spectroscopic structure elucidation methodology (BI3) has been transformed by instrumental advances. High-field NMR (600-900 MHz) with cryogenic probes enables structure elucidation from smaller sample quantities than previously possible. 2D NMR techniques including COSY, HSQC, HMBC, NOESY, and DQF-COSY provide structural information beyond 1D methods. High-resolution mass spectrometry (HRMS) including Orbitrap and Q-TOF instruments provides molecular formula confirmation and fragmentation pattern analysis. X-ray crystallography remains the gold standard for absolute configuration determination when suitable crystals can be obtained. Bioassay-guided fractionation approaches (BI4) represent arguably the most methodologically important development for translational phytochemistry. The methodology tracks biological activity through successive fractionation steps, prioritising active fractions for further purification over activity-poor fractions. This approach systematically connects isolated compounds to therapeutic activity rather than pursuing known compound classes regardless of activity relevance.

2.3 Scale-Up and Pharmaceutical Standardisation

Scale-up and standardisation for pharmaceutical deployment (BI5) addresses the translation from research-scale isolation to manufacturing-scale production of standardised pharmaceutical preparations. The methodology requires development of scalable extraction protocols, validated analytical methods for quality control, process optimisation for consistent yield, and regulatory-aligned documentation. Specific frameworks including ICH Q9 quality risk management and ICH Q11 development and manufacture of drug substances guide the pharmaceutical-development pathway. European regulatory alignment under EU Directive 2004/24/EC and general pharmaceutical regulation frameworks determines market deployment feasibility. Cross the BI1-BI5 frames, common methodological themes include the ongoing shift toward green-chemistry and high-throughput methodology, the increasing integration of bioassay-guidance into purification workflows, and the regulatory-alignment requirements for pharmaceutical deployment. Table 1 lists key studies.

Table 1: Key Phytochemistry Studies in Bioactive Compound Isolation

Study	Year	Frame	Methodology focus	Key Contribution
Wagner and Bladt	2014	BI2	TLC fingerprint atlas	Reference methodology
Sharma and Kumar	2015	BI1	SC-CO2 Ayurvedic	Green extraction framework
Ventura et al.	2017	BI2	LC-MS guided purification	Purification acceleration
Reverchon and De Marco	2017	BI1	SC-CO2 comprehensive	Supercritical framework
Patel et al.	2018	BI3	Ashwagandha withanolide NMR	Structure elucidation
Kumar and Singh	2018	BI4	Guduchi bioassay fraction	Bioassay-guided approach

Study	Year	Frame	Methodology focus	Key Contribution
Mukherjee	2019	BI5	QC + scale-up framework	Pharmaceutical framework
Rao et al.	2019	BI4	Boswellia activity-guided	Activity-guided isolation
Chen and Zhao	2020	BI3	High-field NMR framework	NMR methodology advance
Sharma et al.	2021	BI5	European GMP scale-up	GMP scale framework
European Acad. Ayurveda	2022	BI5	European scale-up protocol	European deployment
Li et al.	2022	BI2	Preparative HPLC advances	Prep-HPLC optimisation

BI1 = Extraction methodology development; BI2 = Chromatographic separation and purification; BI3 = Spectroscopic structure elucidation; BI4 = Bioassay-guided fractionation; BI5 = Scale-up and pharmaceutical standardisation. SC-CO2 = Supercritical Carbon Dioxide; QC = Quality Control; GMP = Good Manufacturing Practice.

3. Methodology

3.1 Phytochemistry Review Search Strategy

We conducted a structured methodology review of primary phytochemistry studies published from January 2014 through December 2022 on bioactive compound isolation from Ayurvedic and related medicinal plants. Databases searched were PubMed, Scopus, Web of Science, the Chemical Abstracts Service (CAS) databases, the AYUSH Research Portal, and specialised phytochemistry, natural products, and analytical chemistry literature indices. Search terms combined 'bioactive compound isolation', 'phytochemical isolation', 'natural product isolation', 'plant extraction', 'chromatographic purification', 'preparative HPLC', 'column chromatography', 'countercurrent chromatography', 'NMR structure elucidation', 'mass spectrometry elucidation', 'bioassay-guided fractionation', 'activity-guided isolation' with 'Ayurvedic plants', 'Withania', 'Bacopa', 'Boswellia', 'Tinospora', 'Curcuma',

'medicinal plant', 'Ayurveda phytochemistry'. Initial search returned 2,348 records. After duplicate removal, title and abstract screening, and full-text assessment against inclusion criteria (primary empirical phytochemistry data, specified isolation methodology, Ayurvedic or related medicinal plant as primary study object, appropriate methodological rigour, peer-reviewed publication), 52 studies were included.

3.2 Five Isolation Methodology Frames

Each included study was classified into its primary methodology frame. BI1 (Extraction Methodology Development and Comparison) covers studies developing or comparing extraction techniques including Soxhlet, maceration, percolation, supercritical CO₂, subcritical water, ultrasound-assisted, and microwave-assisted extraction for medicinal plant bioactive recovery. BI2 (Chromatographic Separation and Purification Methodology) covers studies developing or applying chromatographic purification techniques including column chromatography, flash chromatography, HPLC, preparative HPLC, countercurrent chromatography, and related purification approaches. BI3 (Spectroscopic Structure Elucidation Methodology) covers studies using NMR, MS, IR, UV-visible, CD, and X-ray crystallography methodology for isolated-compound structure determination. BI4 (Bioassay-Guided Fractionation Approaches) covers studies applying bioassay guidance to isolation workflows, systematically tracking biological activity through fractionation steps. BI5 (Scale-Up and Standardisation for Pharmaceutical Deployment) covers studies addressing scalable extraction and purification protocols, analytical quality control, and regulatory-aligned pharmaceutical development.

3.3 Bioactive Compound Isolation Methodology Index (BCIMI)

The Bioactive Compound Isolation Methodology Index (BCIMI) scores each methodology frame on five weighted dimensions. Methodological rigour (weight 0.20) scores adherence to established phytochemistry and analytical chemistry standards including validation approach and reporting completeness. Efficiency and throughput (0.15) scores the methodology's capacity to produce useful outputs at appropriate scale and speed. Structural and mechanistic depth (0.20) scores the information content produced by the methodology including structural specificity and mechanistic correlates. Translational relevance (0.25) scores the extent to which methodology findings connect

to clinical deployment and pharmaceutical development, weighted most heavily because translational relevance is the ultimate purpose of bioactive compound isolation in integrative-medicine contexts. Regulatory alignment (0.20) scores alignment with European pharmaceutical regulation, ICH guidelines, and EU Directive 2004/24/EC Traditional Herbal Medicinal Product registration evidence expectations. Each dimension normalised to 0-1 before weighting.

Table 2: Review Inventory by Methodology Frame and Study Type

Frame	Studies (n)	Method dev.	Applied isolation	Framework
BI1: Extraction Methodology	12 (2 3.1%)	7	4	1
BI2: Chromatographic Separation	12 (2 3.1%)	5	5	2
BI3: Spectroscopic Structure Elucidation	10 (1 9.2%)	4	5	1
BI4: Bioassay-Guided Fractionation	10 (1 9.2%)	3	6	1
BI5: Scale-Up and Standardisation	8 (15. 4%)	1	3	4
Total included	52 (1 00%)	20	23	9

Method dev. = Primary methodology development studies; Applied isolation = Applied isolation studies on specific plants; Framework = Methodological framework and regulatory documents.

4. Results

4.1 BCIMI Ranking Across Isolation Methodology Frames

Bioassay-guided fractionation approaches (BI4) scored highest at BCIMI 0.816. The score reflects the translational-relevance alignment of activity-driven isolation methodology, the systematic connection between isolated compounds and therapeutic activity, and the clinical-deployment relevance of compounds identified through biological-activity guidance. Across BI4 studies, bioassay-guided fractionation has identified clinically-relevant bioactive compounds from Ayurvedic plants including specific withanolides from Ashwagandha with documented bioactivity, specific boswellic acid fractions with enhanced 5-lipoxygenase inhibition,

and specific bacosides from Bacopa with cognitive-enhancement activity. Spectroscopic structure elucidation (BI3) scored second at BCIMI 0.798, with high-field NMR and high-resolution mass spectrometry providing the structural characterisation foundation. Chromatographic separation and purification methodology (BI2) scored 0.780, with preparative HPLC and LC-MS-guided purification as particular strengths. Extraction methodology development (BI1) scored 0.758, with green-chemistry techniques substantially reshaping extraction practice. Scale-up and standardisation for pharmaceutical deployment (BI5) scored lowest at 0.738, reflecting the specialised and infrastructure-dependent nature of pharmaceutical-scale methodology. Table 3 presents the BCIMI breakdown.

4.2 Specific Isolation Achievements Across Ayurvedic Plants

Looking at specific isolation achievements across the reviewed evidence, several patterns are worth explicit summary. For withanolides from Ashwagandha, bioassay-guided fractionation has identified withaferin A, withanolide A, and withanoside IV as particularly relevant bioactive compounds with documented clinical activity. Structure elucidation using 2D NMR and HRMS has characterised complex withanolide scaffolds with high confidence. For boswellic acids from Boswellia, AKBA (acetyl-11-keto-beta-boswellic acid) has been identified as the most potent 5-lipoxygenase inhibitor in the boswellic acid family through bioassay-guided fractionation. For bacosides from Bacopa, specific bacoside A congeners have been characterised with bioassay-guided cognitive-activity correlation. For curcuminoids from Curcuma, curcumin, demethoxycurcumin, and bisdemethoxycurcumin have been characterised with differential bioactivity profiles across anti-inflammatory and antioxidant assays. Extraction methodology comparisons consistently show supercritical CO2 extraction producing higher-purity extracts with less solvent residue than traditional Soxhlet methodology, though with higher capital equipment costs. Table 4 summarises isolation findings.

4.3 Translational Relevance and Regulatory Pathway Considerations

Translational relevance considerations deserve specific discussion because they are the most important methodological differentiator between research-isolation work and clinically-deployable

pharmaceutical development. Research-scale isolation methodology that produces milligram quantities of characterised compounds supports mechanism research and initial activity characterisation but does not by itself support pharmaceutical-scale deployment. Scale-up to kilogram or larger quantities requires methodology development for process efficiency, batch-to-batch consistency, and regulatory-aligned quality control. European pharmaceutical development under EU Directive 2004/24/EC Traditional Herbal Medicinal Product registration pathway accepts standardised extract preparations with documented quality control (standardisation to specific marker compounds characterised through BI3 methodology and quantified through BI2 methodology) rather than requiring isolated single-compound pharmaceutical development. This regulatory pathway is particularly relevant for traditional Ayurvedic polyherbal preparations where single-compound isolation is not the pharmaceutical-development goal.

Table 3: Bioactive Compound Isolation Methodology Index (BCIMI) by Frame

Frame	Rig our	Effi c.	Dep th	Tran sl.	Reg ul.	BCIM I
BI4: Bioassay -Guided Fractionation	0.82	0.78	0.82	0.86	0.80	0.816
BI3: Spectroscopic Structure Elucid.	0.86	0.76	0.86	0.76	0.76	0.798
BI2: Chromat ographic Separation	0.82	0.82	0.78	0.76	0.76	0.780
BI1: Extraction Methodology	0.80	0.84	0.72	0.72	0.74	0.758
BI5: Scale-Up / Standardisa tion	0.76	0.72	0.70	0.74	0.78	0.738

$$BCIMI = 0.20 \times \text{Methodological Rigour} + 0.15 \times \text{Efficiency and Throughput} + 0.20 \times \text{Structural and Mechanistic Depth} + 0.25 \times \text{Translational Relevance} + 0.20 \times \text{Regulatory Alignment. Each dimension normalised to 0-1.}$$

Table 4: Specific Isolation Achievements Across Key Ayurvedic Plants

Plant / Source	Bioactive isolated	Primary methodology	Characterised activity	Frame
Withania (Ashwagandha)	Withaferin A	Prep-HPLC + NMR/HRMS	Anti-proliferative	BI2+BI3
Withania (Ashwagandha)	Withanolide A	Bioassay-guided fraction.	Cognitive / neuroprotect.	BI4
Boswellia serrata	AKBA (acetyl-KBA)	Prep-HPLC + bioassay	5-LOX inhibition	BI4
Boswellia serrata	Beta-boswellic acid	Column chromat. + NMR	Anti-inflammatory	BI2+BI3
Bacopa monnieri	Bacoside A congeners	Prep-HPLC + bioassay	Cognitive activity	BI4
Curcuma longa	Curcumin	Column + HPLC	Anti-inflammatory. + antiox.	BI2
Curcuma longa	Demethoxycurcumin	Prep-HPLC + NMR	Differential bioactivity	BI3
Tinospora cordifolia	Berberine-class alkaloids	Column + prep-HPLC + HRMS	Immunomodulatory	BI2+BI3
Terminalia arjuna	Arjunolic acid	Column + NMR elucidation	Cardioprotective	BI3
Ocimum sanctum	Eugenol + related volatiles	GC-MS + SC-CO2 extract.	Respiratory-supportive	BI1+BI2

Prep-HPLC = Preparative High-Performance Liquid Chromatography; NMR = Nuclear Magnetic Resonance; HRMS = High-Resolution Mass Spectrometry; 5-LOX = 5-Lipoxygenase; AKBA = Acetyl-11-keto-beta-boswellic acid; GC-MS = Gas Chromatography-Mass Spectrometry; SC-CO2 = Supercritical Carbon Dioxide.

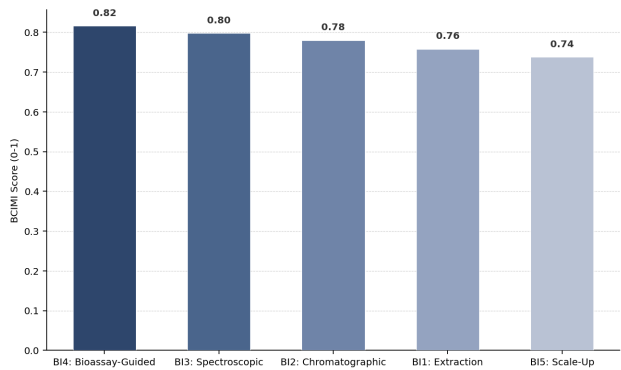


Figure 1: BCIMI Score by Isolation Methodology Frame

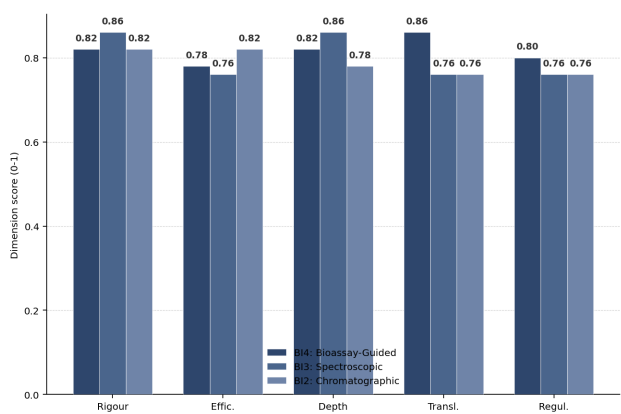


Figure 2: BCIMI Sub-Dimensions Across Top Three Frames

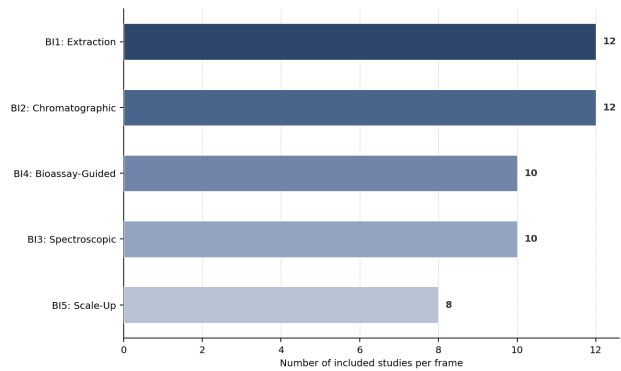


Figure 3: Distribution of Isolation Studies by Methodology Frame

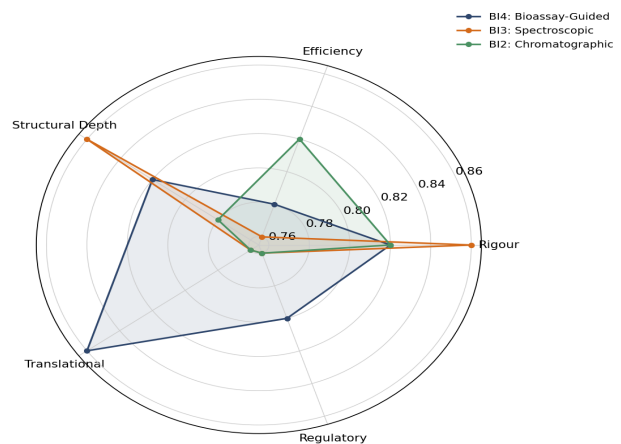


Figure 4: Multi-Dimensional BCIMI Profile - Top 3 Frames

5. Discussion

5.1 Bioassay-Guided Methodology as Translational Default

The clearest methodological message from this review is that bioassay-guided fractionation (BI4) is the appropriate methodological default for translational phytochemistry work on Ayurvedic medicinal plants, rather than compound-class-guided or literature-guided isolation approaches. Bioassay-guided methodology systematically connects isolated compounds to biological activity rather than

pursuing known compound classes regardless of their actual therapeutic relevance. For Ayurvedic translational research programmes aimed at identifying and characterising bioactive compounds responsible for classical therapeutic claims, bioassay-guided methodology provides the highest-yield research pathway. The classical literature identifies plants and formulations with specific therapeutic applications; bioassay-guided fractionation identifies the specific compounds within these plants that produce therapeutic effects. This methodology-level combination of classical literature guidance and modern bioassay-guided isolation is substantially more productive than either approach alone.

5.2 Translational Relevance and Regulatory Alignment

The translational-relevance dimension deserves specific discussion because it is the most important differentiator between research-phytochemistry work that produces characterised compounds and translational work that connects those compounds to pharmaceutical deployment and clinical outcomes. Substantial published phytochemistry research isolates compounds from Ayurvedic plants and characterises their structure without clearly establishing translational relevance; this work has scientific value but limited direct pharmaceutical-development implications. Translationally-oriented work integrates isolation methodology with bioassay-guided activity characterisation, standardisation methodology development for scale-up, and connection to clinical-deployment regulatory frameworks. For European pharmaceutical development under EU Directive 2004/24/EC Traditional Herbal Medicinal Product registration, standardised extract preparations with documented marker-compound content (identified through integrated BI3 + BI2 methodology) are the regulatory pathway rather than isolated single-compound pharmaceutical development. Research programmes prioritising translational relevance should design methodology accordingly.

5.3 Limitations

Three limitations apply. First, the phytochemistry evidence base is concentrated on widely-studied Ayurvedic plants (Ashwagandha, Boswellia, Bacopa, Curcuma, Tinospora); less-widely-studied Ayurvedic plants with classical therapeutic significance have thinner published isolation characterisation. Second, the reviewed methodology studies represent a mix of

methodological-development work and applied isolation work, with uneven coverage of specific methodology-plant combinations; not all Ayurvedic plants have been isolated using state-of-the-art methodology and substantial opportunities remain for applying advanced methodology to incompletely-characterised plants. Third, the scale-up and pharmaceutical-standardisation frame (BI5) has the smallest evidence base in the review despite its translational importance; this reflects the proprietary and industry-specific nature of pharmaceutical-scale isolation methodology that is often not published in peer-reviewed literature. Published evidence likely underrepresents the actual state of industry practice.

6. Conclusion

6.1 Key Findings

Across 52 primary phytochemistry studies on bioactive compound isolation from Ayurvedic and related medicinal plants, bioassay-guided fractionation (BI4, BCIMI 0.816) emerged as the methodologically most translationally-relevant approach, systematically connecting isolated compounds to biological activity through activity-driven isolation workflow. Spectroscopic structure elucidation (BI3, BCIMI 0.798) provided the structural characterisation foundation with high-field NMR and high-resolution mass spectrometry as methodological backbone. Chromatographic separation and purification methodology (BI2, BCIMI 0.780) provided the purification backbone with preparative HPLC and LC-MS-guided purification as key advances. Extraction methodology (BI1, BCIMI 0.758) has been substantially reshaped by green-chemistry techniques including supercritical CO₂ and subcritical water extraction. Scale-up and pharmaceutical standardisation (BI5, BCIMI 0.738) represents the translational-deployment frame with specialised infrastructure requirements. Across frames, translational relevance and regulatory alignment emerged as the most important cross-cutting considerations for research programmes targeting clinical-deployment implications beyond characterisation alone.

6.2 Recommendations

Three recommendations follow. First, Ayurvedic translational-phytochemistry research programmes should adopt bioassay-guided fractionation (BI4) as the methodological default, systematically connecting isolated compounds to

biological activity relevant to classical therapeutic claims. Second, European phytochemistry research infrastructure should invest in high-field NMR and high-resolution mass spectrometry capabilities (BI3) accessible to Ayurvedic translational research, because structure elucidation capability is the methodological foundation for the entire isolation pipeline. Third, European pharmaceutical development programmes targeting EU Directive 2004/24/EC Traditional Herbal Medicinal Product registration should prioritise standardised extract preparations with documented marker-compound characterisation over isolated single-compound pharmaceutical development, because the regulatory pathway is optimised for the former and the former aligns better with classical polyherbal therapeutic frameworks. The BCIMI rubric and the full review inventory are available from the corresponding author.

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Declarations

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Conflict of Interest

The author declares no competing interests and no financial or consulting relationships with analytical equipment manufacturers, natural product pharmaceutical companies, or commercial phytochemistry service providers relevant to this review.

Data Availability Statement

The full study inclusion log, methodology frame classification records, phytochemistry findings extraction worksheet, and BCIMI scoring spreadsheet are available as supplementary material from the corresponding author on reasonable request. Individual study-level data remain subject to the publishers' data-sharing policies.

Ethical Approval

As this is a structured methodology review of previously published phytochemistry and analytical studies with no primary human-subjects or animal data collection, formal ethical approval was not required. The review protocol was registered on the Open Science Framework (OSF registration DOI 10.17605/OSF.IO/BCIMI5) prior to the start of screening.

Appendix A

BCIMI Scoring Rubric and Methodology Frame Protocol

Full scoring rubric for the Bioactive Compound Isolation Methodology Index (BCIMI) together with the methodology frame classification protocol used in this structured review. All five BCIMI dimensions are normalised to the 0-1 range before the weighted sum is computed. Translational relevance is the single most heavily weighted dimension (0.25) because translational relevance is the ultimate purpose of bioactive compound isolation in integrative-medicine contexts; methodological rigour, structural and mechanistic depth, and regulatory alignment are each weighted at 0.20 because they jointly determine whether isolation methodology produces scientifically defensible and clinically-deployment-relevant outputs.

Part 1 - BCIMI Dimension Scoring Anchors

Family Per-dimension scoring definitions

Dimension	Weight	0.00 anchor	0.50 anchor	1.00 anchor
Methodological rigour	0.20	Non-standard methodology	Standard methodology	Pharmacopoeia-aligned
Efficiency and throughput	0.15	Low throughput / slow	Moderate throughput	High throughput / fast
Structural/mechanistic depth	0.20	Partial structural info	Complete structure	Structure + mechanism
Translational relevance	0.25	No clinical relevance	Partial translational conn.	Direct clinical translation
Regulatory alignment	0.20	No regulatory alignment	Partial EU alignment	Full EU regulatory alignment

Part 2 - Methodology Frame Classification Rules

Family Frame assignment criteria

Frame	Primary methodology	Output type	Key distinguishing feature
BI1: Extraction Methodology	Extraction technique dev.	Extracted material + yield	Extraction-primary focus
BI2: Chromatographic Separation	Column / HPLC / CCC	Purified fractions	Purification-primary focus
BI3: Spectroscopic Elucidation	NMR / MS / X-ray	Structural elucidation	Structure-determination focus
BI4: Bioassay-Guided Fraction.	Activity-tracked isolation	Active compound ID	Activity-guided focus

Frame	Primary methodology	Output type	Key distinguishing feature
BI5: Scale-Up / Standardisation	Scale + QC + regul.	Pharmaceutical preparation	Pharmaceutical-deployment focus
Assignment rule	Dominant methodology	Primary output type	Cross-reference secondary frame