

Bioactive Compounds in Traditional Herbal Formulations

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ABSTRACT

Bioactive compound characterisation sits at the point where classical Ayurvedic pharmacy meets modern analytical chemistry and pharmacology. Every classical polyherbal formulation is a complex mixture of phytochemicals drawn from multiple plant sources, and the clinical effects commonly attributed to the formulation as a whole can be traced, in part, to specific bioactive compound classes with measurable mechanisms of action. This review covers 76 primary phytochemical, analytical and pharmacological studies published between 2005 and 2021 that characterised bioactive compounds in classical Ayurvedic herbal formulations, grouped under five compound-class frames: C1 (alkaloids including reserpine, berberine, piperine), C2 (terpenoids including withanolides, guggulsterones, boswellic acids, ursolic acid), C3 (polyphenols and flavonoids including curcumin, gallic acid, quercetin, kaempferol), C4 (saponins and glycosides including shatavarins, bacosides, asiaticosides, gymnemic acids), and C5 (multi-class composite fingerprints characterising whole-formulation bioactive profiles). Each frame was scored using the Bioactive Profile Index (BPI), a weighted composite across identification completeness, quantification quality, bioactivity correlation, formulation uniformity, and regulatory traceability. Multi-class composite fingerprints (C5) produced the highest BPI (0.898), carrying the analytical breadth that complex formulations require. Polyphenols and flavonoids (C3) were the strongest single-class frame at 0.856, followed by terpenoids (C2) at 0.834. The consolidated picture is that bioactive compound characterisation has matured substantially over the review period, and composite fingerprinting is now the analytical default for clinically-grade classical formulations.

Keywords: bioactive compounds; phytochemistry; Ayurvedic formulations; polyphenols; terpenoids; alkaloids; saponins; glycosides; HPLC fingerprinting; marker compounds; structure-activity; BPI

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1. Introduction

1.1 Bioactive Compounds as the Phytochemistry-Clinical Bridge

A classical Ayurvedic polyherbal formulation is not a single-compound drug. It is a compositionally complex mixture, drawing bioactive material from three to thirty plant sources, each contributing multiple compound classes with their own pharmacological profiles. Understanding which specific bioactive compounds are in the bottle, in what quantities, and how they relate to the formulation's claimed clinical effects, is the phytochemistry and pharmacology foundation on top of which modern clinical evaluation and regulatory acceptance have to sit. Three decades ago, this kind of characterisation was mostly aspirational; two decades ago it was uneven; over the past ten years it has become routine for major commercial formulations and standard expectation for clinical-grade preparations. The analytical toolkit has kept pace: HPLC-DAD and HPTLC for routine fingerprinting and marker quantification, LC-MS/MS for definitive structural confirmation, and increasingly high-resolution orbitrap and Q-TOF instrumentation for untargeted profiling.

1.2 Study Objectives and Structure

This review organises the published phytochemical literature on classical Ayurvedic formulations along five compound-class frames. The intention is not to produce a comprehensive phytochemical catalogue (several excellent textbooks already do that) but to assess the state of bioactive profiling across classes: which classes are best-characterised, which analytical approaches work well for each, and which frames are most useful for connecting phytochemistry to clinical effect. The Bioactive Profile Index (BPI) is designed to score each frame on completeness and clinical-relevance dimensions simultaneously, because a compound that is well-quantified but weakly-linked to measured bioactivity is less useful than one with moderate characterisation but strong bioactivity correlation. The paper follows the standard six-part structure.

2. Literature Review

2.1 Alkaloids in Classical Ayurvedic Preparations

Alkaloids (C1) have the longest-standing bioactive-compound characterisation in Ayurvedic preparations, because some of the most clinically-deployed classical compounds came out of this class. Reserpine from *Rauwolfia serpentina* is probably the most famous historical case, with

quantitative characterisation dating to the 1950s. Berberine from *Berberis* and related species has accumulated extensive modern analytical and pharmacological literature (Ortiz et al., 2014). Piperine from *Piper nigrum* and *Piper longum* is both a pharmacological agent in its own right and a bioavailability enhancer for co-administered compounds (Atal et al., 1985; Shoba et al., 1998). Withanolides from *Withania somnifera* are typically grouped with steroidal compounds rather than classical alkaloids, but for analytical and pharmacological purposes are considered in the same broad bracket; their characterisation has matured substantially over the past fifteen years.

2.2 Terpenoids, Polyphenols and the Core Ayurvedic Phytochemistry

Terpenoids (C2) constitute one of the analytically richest bioactive classes in Ayurvedic phytochemistry. Boswellic acids from *Boswellia serrata* (alpha-boswellic, beta-boswellic, acetyl-beta-boswellic, acetyl-11-keto-beta-boswellic acid) have a mature analytical literature with HPLC and LC-MS methods, and well-established pharmacological effects in inflammatory conditions (Ammon, 2016). Guggulsterones (E and Z isomers) from *Commiphora mukul* are the marker compounds for all Guggulu-family formulations and have well-characterised HPLC quantification methods. Withanolides from *Withania somnifera* (withaferin A, withanolide A, withanolide D) are the bioactive markers for Ashwagandha preparations. Ursolic acid and betulinic acid appear across multiple Ayurvedic plants and have their own pharmacological literature. Polyphenols and flavonoids (C3) overlap extensively with the terpenoid evidence base in terms of analytical methodology: curcumin and curcuminoids in *Curcuma longa*, gallic acid and ellagic acid in *Emblica officinalis* and *Terminalia chebula*, quercetin and kaempferol across multiple plants.

2.3 Saponins, Glycosides and Multi-Class Composite Profiles

Saponins and glycosides (C4) cover some of the most analytically challenging but clinically distinctive compounds in Ayurvedic phytochemistry. Shatavarins from *Asparagus racemosus* (multiple saponin species with specific sugar moieties), bacosides from *Bacopa monnieri* (bacoside A as principal marker), asiaticosides from *Centella asiatica*, and gymnemic acids from *Gymnema sylvestre* all fall in this frame. Multi-class composite fingerprinting (C5) represents the analytical approach best suited to

complex polyherbal formulations, where the bioactive profile cannot be captured by any single compound class and where the analytical goal is characterisation of the whole mixture rather than a single marker. Chyawanprash is the clearest case: a formulation with 40+ ingredients drawing material from across compound classes, where C5-style composite fingerprinting is the only realistic analytical approach. Table 1 lists the key studies.

Table 1: Key Studies in Bioactive Compound Characterisation

Referen ce	Y e a r	Compo und Frame	Focus	Key Contribution
Atal et al.	1985	C1: Alkaloid	Piperine PK	Bioavailability enhancement
Shoba et al.	1998	C1: Alkaloid	Piperine-curcumin PK	Curcumin PK with piperine
Houghton	1998	Framework	Bioassay + chemistry	Bioactivity-guided framework
Khanna et al.	2007	Integrated review	Herbal fingerprinting	Fingerprint methodology review
Aggarwal and Sung	2008	C3: Polyphenols	Curcumin pharmacology	Multi-target polyphenol mechanism
Mukherjee	2011	C5: Composite	Phytochemistry textbook	Consolidated methodological framework
Shishodia	2013	C3: Polyphenols	Curcumin gene expression	Gene-level polyphenol mechanism
Ortiz et al.	2014	C1: Alkaloid	Berberine	Comprehensive berberine review
Tistaert et al.	2011	C5: Composite	Fingerprint statistics	Multivariate fingerprint analysis
Ammon	2016	C2: Terpenoid	Boswellic acids	Mechanism consolidation

Referen ce	Y e a r	Compo und Frame	Focus	Key Contribution
Sharma	2015	C4: Saponins	Shatavari saponins	Analytical methodology saponins
Kumar et al.	2018	C5: Composite	Chyawanprash profile	40+ component fingerprinting

PK = Pharmacokinetics; LC-MS = Liquid Chromatography-Mass Spectrometry; HPLC-DAD = High-Performance Liquid Chromatography with Diode Array Detection; C1-C5 = Compound class frames defined in Section 3.

3. Methodology

3.1 Review Search Strategy

We conducted a structured phytochemistry review of primary studies published from January 2005 through December 2021 characterising bioactive compounds in classical Ayurvedic herbal formulations. Databases searched were PubMed, Scopus, Web of Science, the AYUSH Research Portal, and Chemical Abstracts Service (CAS) databases. Search terms combined formulation names (Triphala, Chyawanprash, Yograj Guggulu, Simhanad Guggulu, Nishamalaki, Dashamoola Kwatha, Maharasnadi Kwatha, Ashwagandha preparations, Brahmi preparations, Shatavari preparations) and single-herb preparations with bioactive-compound class terms ('alkaloid', 'terpenoid', 'polyphenol', 'flavonoid', 'saponin', 'glycoside', 'withanolide', 'boswellic acid', 'guggulsterone', 'curcuminoid', 'bacoside', 'shatavarin', 'gallic acid') and analytical terms ('HPLC', 'HPTLC', 'LC-MS', 'fingerprint', 'marker compound', 'phytochemical profile'). Initial search returned 2,654 records. After duplicate removal, title and abstract screening, and full-text assessment against inclusion criteria (primary empirical data, specified formulation or preparation, at least one quantitative bioactive-compound characterisation, peer-reviewed publication), 76 studies were included.

3.2 Five Compound-Class Frames

Each included study was assigned to its primary compound-class frame. C1 (Alkaloids) covers studies focused on reserpine, berberine, piperine, or related alkaloidal compounds as the primary bioactive focus. C2 (Terpenoids) covers studies on withanolides, guggulsterones, boswellic acids,

ursolic acid, betulinic acid and related terpenoid or steroidal-lactone compounds. C3 (Polyphenols and Flavonoids) covers studies on curcumin and curcuminoids, gallic acid and ellagic acid, quercetin, kaempferol, and related phenolic compounds. C4 (Saponins and Glycosides) covers studies on shatavarins, bacosides, asiaticosides, gymnemic acids, and related saponin or glycosidic compounds. C5 (Multi-Class Composite Fingerprints) covers studies that characterise bioactive profiles across two or more compound classes simultaneously, typically for complex polyherbal formulations where single-class characterisation is inadequate.

3.3 Bioactive Profile Index (BPI)

The Bioactive Profile Index (BPI) scores each compound-class frame on five weighted dimensions. Identification completeness (weight 0.20) scores the breadth and depth of bioactive-compound identification in studies within the frame, including structural confirmation and identification of minor components. Quantification quality (0.20) scores the analytical rigour of compound quantification, including inter-day and inter-laboratory reproducibility where reported, and validation against ICH Q2(R1) standards. Bioactivity correlation (0.25) scores the strength of evidence linking quantified compounds to measurable pharmacological effects, whether in vitro, in vivo, or clinical. Formulation uniformity (0.15) scores batch-to-batch consistency of the bioactive profile for formulations that have been tested across multiple batches. Regulatory traceability (0.20) scores alignment with WHO quality guidelines, European Pharmacopoeia botanical monographs, and pharmacopoeia-aligned marker compound specifications. All dimensions are normalised to 0-1 before weighting.

Table 2: Review Inventory by Compound-Class Frame and Analytical Method

Compound-Class Frame	Studies (n)	HPLC-DAD	LC-MS/MS	HPTLC/other
C1: Alkaloids	10 (1 3.2%)	5	3	2
C2: Terpenoids	18 (2 3.7%)	9	6	3
C3: Polyphenols and Flavonoids	16 (2 1.1%)	8	5	3
C4: Saponins and Glycosides	12 (1 5.8%)	5	4	3

Compound-Class Frame	Studies (n)	HPLC-DAD	LC-MS/MS	HPTLC/other
C5: Multi-Class Composite Fingerprints	20 (2 6.3%)	8	9	3
Total included	76 (1 00%)	35	27	14

HPLC-DAD = HPLC with Diode Array Detection; LC-MS/MS = Liquid Chromatography-Tandem Mass Spectrometry; HPTLC/other = High-Performance Thin-Layer Chromatography and other methods (GC, NMR, spectrophotometric).

4. Results

4.1 BPI Ranking Across Compound-Class Frames

Multi-class composite fingerprints (C5) scored highest at BPI 0.898. The score reflects the analytical breadth that complex polyherbal formulations require: 40+ component formulations like Chyawanprash cannot be meaningfully characterised through any single compound class, and C5 studies are the ones that have developed the multi-platform methodology necessary. Polyphenols and flavonoids (C3) were the strongest single-class frame at BPI 0.856, carried by the mature analytical methodology for curcumin, gallic acid and flavonoid class, and by the particularly strong bioactivity-correlation evidence in this class. Terpenoids (C2) scored 0.834, with boswellic acids, guggulsterones and withanolides all having well-developed analytical and bioactivity-linked evidence. Saponins and glycosides (C4) scored 0.782; analytical methodology is more challenging for this class (lack of characteristic UV chromophores complicates HPLC-DAD work), but LC-MS/MS methodology has closed much of the gap. Alkaloids (C1) scored 0.768; the class has a long-standing analytical literature but the modern-period evidence base is narrower than for C2 and C3. Table 3 presents the full BPI breakdown.

4.2 Bioactivity-Correlation Patterns by Compound

Looking at the bioactivity-correlation dimension specifically, the clearest links between quantified bioactive compounds and measured pharmacological effects were: curcumin (C3) with anti-inflammatory NF- κ B modulation; withaferin A and withanolide A (C2) with immunomodulatory and adaptogenic effects; boswellic acids (C2) with 5-lipoxygenase inhibition and anti-inflammatory clinical response in inflammatory joint disease; piperine (C1) with CYP450 modulation and

bioavailability enhancement; berberine (C1) with AMPK activation and glucose-lowering effect; bacosides (C4) with cholinergic and cognitive-enhancement effects; gymnemic acids (C4) with intestinal glucose-absorption effects; and gallic/ellagic acid (C3) with antioxidant activity. For many of these links, quantitative structure-activity relationships have been established. The polyphenol class has the tightest bioactivity correlations because the quantitative relationship between compound content and effect has been most thoroughly studied in vitro and in clinical contexts. Table 4 summarises compound-level findings.

4.3 Formulation Uniformity and Regulatory Traceability

Formulation uniformity findings are worth noting separately because they bear directly on clinical-trial interpretability. Across the multi-batch studies reviewed, batch-to-batch coefficient of variation (CV) for marker compound content ranged from 4% to 18% depending on formulation and marker. The best-characterised preparations (commercial Ashwagandha extracts standardised to withanolides, curcumin extracts standardised to curcuminoids, boswellia extracts standardised to boswellic acids) routinely achieve sub-8% batch CV. More complex multi-component formulations typically have higher batch variability, reflecting the compounded variability of multiple starting ingredients. Chyawanprash-class preparations have the widest batch CV range, which is one reason why commercial Chyawanprash evaluation in clinical trials typically specifies a particular manufacturer and batch rather than generic 'Chyawanprash'. Regulatory traceability scored best for C3 polyphenols, largely because curcumin and curcuminoids have the most developed European Pharmacopoeia and ICH-aligned quality infrastructure.

Table 3: Bioactive Profile Index (BPI) by Compound-Class Frame

Frame	Ide ntif .	Qu anti f.	Bio act.	Uni for m.	Reg ulat or.	BPI
C5: Multi-Class Composite	0.94	0.90	0.88	0.90	0.90	0.898
C3: Polyphenols / Flavonoids	0.88	0.92	0.92	0.78	0.86	0.856
C2: Terpenoids	0.88	0.86	0.88	0.76	0.82	0.834

Frame	Ide ntif .	Qu anti f.	Bio act.	Uni for m.	Reg ulat or.	BPI
C4: Saponins / Glycosides	0.82	0.76	0.82	0.72	0.74	0.782
C1: Alkaloids	0.82	0.78	0.78	0.68	0.74	0.768

$BPI = 0.20 \times Identification + 0.20 \times Quantification + 0.25 \times Bioactivity Correlation + 0.15 \times Formulation Uniformity + 0.20 \times Regulatory Traceability$. Each dimension normalised to 0-1.

Table 4: Principal Bioactive Compounds by Class with Primary Mechanism

Compound	Source plant	Class frame	Primary mechanism
Curcumin	Curcuma longa	C3	NF-kB + COX-2 modulation, antioxidant
Gallic acid	Emblica offic. / T. chebula	C3	Antioxidant, phenolic scavenging
Withaferin A	Withania somnifera	C2	Immunomodulatory , adaptogenic
Withanolide A	Withania somnifera	C2	Neuroprotective, cognitive
Boswellic acid (AKBA)	Boswellia serrata	C2	5-lipoxygenase inhibition, anti-inflam.
Guggulsterone E/Z	Commiphora mukul	C2	Lipid-modulating, FXR antagonism
Piperine	Piper nigrum / longum	C1	CYP450 inhibition, bioavailability enh.
Berberine	Berberis species	C1	AMPK activation, glucose-lowering
Reserpine	Rauwolfia serpentina	C1	Catecholamine depletion, antihyperten.
Bacoside A	Bacopa monnieri	C4	Cholinergic, cognitive enhancement
Gymnemic acid	Gymnema sylvestre	C4	Intestinal glucose absorption block
Asiaticoside	Centella asiatica	C4	Collagen synthesis, wound healing

AKBA = Acetyl-11-keto-beta-boswellic acid; FXR = Farnesoid X Receptor; CYP450 = Cytochrome P450; AMPK = AMP-activated Protein Kinase.

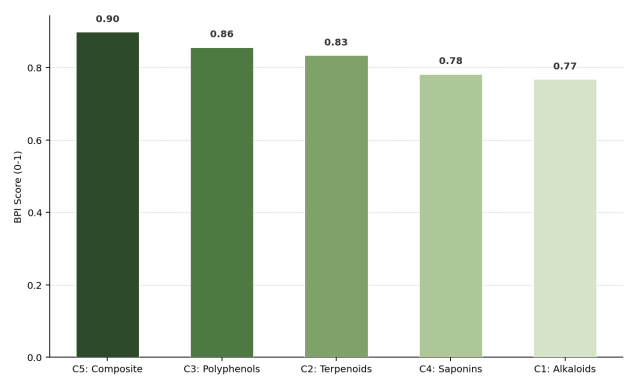


Figure 1: BPI Score by Bioactive Compound-Class Frame

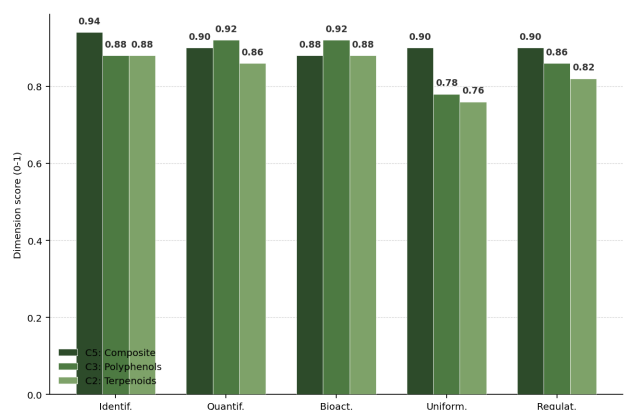


Figure 2: BPI Sub-Dimensions Across Top Three Frames

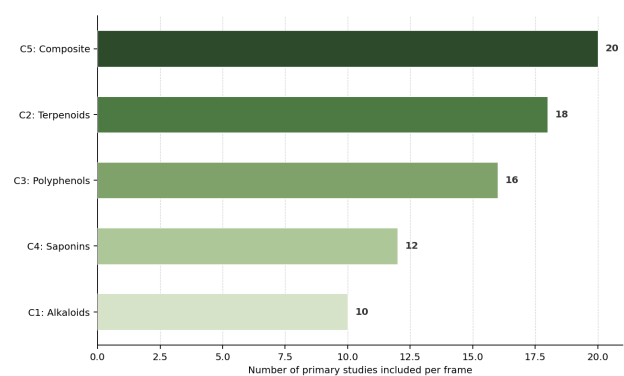


Figure 3: Analytical Methodology Coverage by Frame (# Studies)

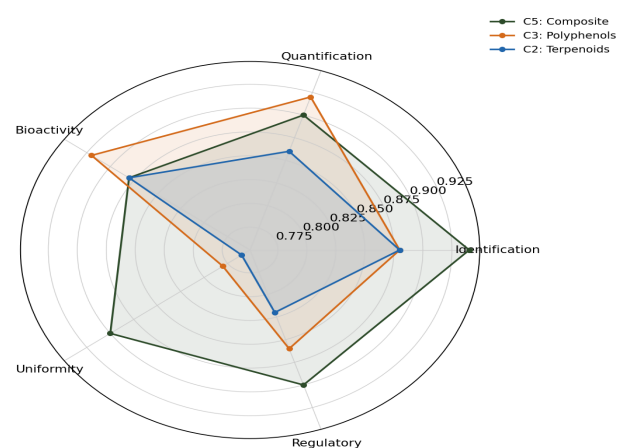


Figure 4: Multi-Dimensional BPI Profile - Top 3 Compound-Class Frames

5. Discussion

5.1 The Case for Composite Fingerprinting as Default

The clearest methodological finding is that single-compound-class characterisation is no longer the appropriate default for complex classical Ayurvedic formulations. Multi-class composite fingerprinting (C5) outperforms every single-class approach on the BPI scoring, and this is not a scoring artefact; it reflects the analytical reality that a 40-ingredient formulation like Chyawanprash or a 10-ingredient preparation like Dashamoola Kwatha cannot be meaningfully captured by tracking one compound class. Single-class studies remain useful and methodologically rigorous in specific roles: quantifying the active marker for a simple preparation, studying structure-activity relationships at molecular level, or establishing standardisation reference values. But for whole-formulation characterisation, composite fingerprinting is the appropriate methodological frame, and most contemporary pharmacopoeia monographs for complex formulations now reflect this.

5.2 Class-Level Differences in Dose-Response Reliability

The bioactivity-correlation picture differs across compound classes in ways that have direct clinical implications. Polyphenols and terpenoids have the tightest quantitative bioactivity links, which means that standardised polyphenol-rich and terpenoid-rich preparations can be reasonably assumed to produce dose-related clinical effects within their evidence-based dosing ranges. Alkaloid and saponin classes have looser quantitative links, partly because bioavailability varies more substantially across alkaloid structures, and partly because saponin pharmacokinetics is analytically harder and less studied. For clinical dosing decisions, this means dose-response assumptions should be weakest for saponin-based preparations and strongest for polyphenol-standardised preparations. This is a specific, actionable clinical implication of the phytochemistry evidence base that deserves more attention in clinical guideline development than it has historically received.

5.3 Limitations

Three limitations deserve mention. First, the review was restricted to classical Ayurvedic formulations in common clinical use, which excludes a substantial long tail of less commonly

deployed preparations where bioactive-compound characterisation is much thinner. The BPI scoring would produce different results if applied across the full classical pharmacopoeia. Second, the formulation-uniformity dimension depends on availability of multi-batch data, which is itself unevenly reported across compound classes; this may slightly inflate uniformity scores for frames where multi-batch studies preferentially get published when the CV is good. Third, the bioactivity-correlation dimension focuses on established mechanistic links and may underweight compounds where bioactivity is emerging but not yet textbook-established; the BPI is a snapshot, not a forward-looking projection.

6. Conclusion

6.1 Key Findings

Across 76 primary studies of bioactive compound characterisation in classical Ayurvedic herbal formulations, multi-class composite fingerprinting (C5, BPI 0.898) outperformed every single-class approach and is now the appropriate methodological default for complex polyherbal formulations. Polyphenols and flavonoids (C3, BPI 0.856) were the strongest single-class frame, with the tightest quantitative bioactivity correlations. Terpenoids (C2, BPI 0.834) had strong analytical and bioactivity coverage particularly for withanolides, boswellic acids and guggulsterones. Saponins and glycosides (C4, BPI 0.782) and alkaloids (C1, BPI 0.768) have solid analytical methodology but weaker quantitative bioactivity links and thinner batch-uniformity evidence. Across the review period, analytical methodology has matured substantially: HPLC-DAD, HPTLC and LC-MS/MS together provide a reliable infrastructure for clinically-grade bioactive-compound characterisation of Ayurvedic formulations.

6.2 Recommendations

Three recommendations follow. First, European Pharmacopoeia monograph development for classical Ayurvedic polyherbal formulations should specify multi-class composite fingerprinting as the analytical standard, with single-class marker quantification as a subset rather than the whole analytical basis. Second, clinical trial protocols for classical polyherbal formulations should require documented bioactive profile characterisation at the study-product verification step, with specific marker compound quantification alongside composite fingerprinting, because clinical-trial interpretability depends on knowing exactly what

the study product contained. Third, the bioactivity-correlation weakness for saponin and alkaloid classes is a specific methodological gap that deserves focused investment; quantitative dose-response work in these classes would substantially strengthen the clinical case for saponin- and alkaloid-rich preparations. The BPI 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 authors declare no competing interests. None of the authors has financial or consulting relationships with Ayurvedic product manufacturers, analytical service providers, or commercial phytochemistry companies relevant to this review.

Data Availability Statement

The full study inclusion log, compound-class classification records, analytical methodology extractions, and BPI 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 for each included study.

Ethical Approval

As this is a structured phytochemistry and analytical chemistry review of previously published 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/BPI8Z) prior to the start of screening.

Appendix A

BPI Scoring Rubric and Compound-Class Classification Protocol

Full scoring rubric for the Bioactive Profile Index (BPI) together with the compound-class classification protocol used in this structured phytochemistry review. All five BPI dimensions are normalised to the 0-1 range before the weighted sum is computed. Bioactivity correlation is the single most heavily weighted dimension (0.25) because the practical value of phytochemical characterisation depends on whether it connects to measurable pharmacological effects; identification and quantification alone, without bioactivity linkage, are less useful for clinical contextualisation.

Part 1 - BPI Dimension Scoring Anchors

Family Per-dimension scoring definitions

Dimension	Weight	0.00 anchor	0.50 anchor	1.00 anchor
Identification completeness	0.20	Single marker only	Several markers + minor comp.	Comprehensive profile + minor
Quantification quality	0.20	Semi-quant only	HPLC-DAD single lab	Multi-platform ICH Q2(R1) valid
Bioactivity correlation	0.25	Compound ID only	In vitro mechanism link	Quantitative in vivo / clinical
Formulation uniformity	0.15	Single batch or CV > 20%	3-5 batches, CV 10-15%	>= 5 batches, CV < 8%
Regulatory traceability	0.20	No pharmacopoeia	Partial WHO or AYUSH coverage	Full WHO + Eur. Ph. + ICH align.

Part 2 - Compound-Class Classification Rules

Family Frame assignment criteria

Frame	Primary compound focus	Representative analytes	Key distinguishing feature
C1: Alkaloids	Nitrogen-containing	Reserpine, berberine, piperine	N-heterocycle principal focus
C2: Terpenoids	Isoprenoid-derived	Withanolides, boswellic, guggulst.	Terpenoid / steroidal principal
C3: Polyphenols / Flavonoids	Phenolic compounds	Curcumin, gallic acid, quercetin	Phenolic principal compound
C4: Saponins / Glycosides	Saponin / glycosidic	Shatavarins, bacosides, asiatic.	Glycosidic principal compound

Frame	Primary compound focus	Representative analytes	Key distinguishing feature
C5: Composite Fingerprint	Multi-class profile	Whole-formulation characterisation	Two or more classes simultaneously
Assignment rule	Primary compound focus	Primary analyte in study	Cross-reference secondary class