

Pharmacognostic Analysis of Medicinal Plants

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ABSTRACT

Pharmacognostic analysis of medicinal plants sits at the methodological foundation of any credible clinical or pharmaceutical programme involving plant-derived interventions. It establishes identity, rules out adulterants, verifies authenticity, and provides the reference physicochemical baseline against which manufacturing quality can be checked. For Ayurvedic medicinal plants entering European pharmaceutical and clinical frameworks, pharmacognostic characterisation is the entry gate through which regulatory acceptance passes. This study reviews 72 primary pharmacognostic analysis studies published between 2005 and 2021 covering the principal Ayurvedic medicinal plants in current clinical and commercial use (Withania somnifera, Curcuma longa, Ocimum sanctum, Tinospora cordifolia, Terminalia chebula, Emblica officinalis, Asparagus racemosus, Boswellia serrata and analogues). Studies are grouped under five analysis frames: P1 (macroscopic and organoleptic examination), P2 (microscopic and anatomical characterisation), P3 (physicochemical analysis including moisture, ash and extractive values), P4 (advanced instrumental analysis using HPLC, HPTLC, LC-MS and GC), and P5 (integrated multi-method pharmacognostic systems including DNA barcoding). Each frame is scored with the Pharmacognostic Analysis Standardisation Index (PASI), a weighted composite across identity accuracy, reproducibility, regulatory alignment, deployability for routine quality control, and discrimination of adulterants. Integrated multi-method systems (P5) scored highest at PASI 0.908, with advanced instrumental analysis (P4) as the strongest single-method frame at 0.844. The practical finding is that DNA barcoding has crossed the threshold from research tool to routine quality control utility, and should be regarded as a minimum component of any credible pharmacognostic system for powdered or processed Ayurvedic raw materials.

Keywords: pharmacognosy; medicinal plants; Ayurveda; HPLC; HPTLC; microscopy; DNA barcoding; adulteration; plant authentication; quality control; pharmacopoeia; PASI

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1. Introduction

1.1 Pharmacognostic Analysis and the Regulatory Gate

Pharmacognosy is the older sister of pharmaceutical chemistry. Before there were clean synthesis routes and pure active ingredients, identifying and authenticating medicinal plants was the fundamental pharmaceutical problem. Modern pharmacognostic analysis has inherited that fundamental role but now operates with a vastly richer toolkit: classical macroscopic and microscopic characterisation, now joined by high-performance chromatographic fingerprinting, mass-spectrometric identity confirmation, and DNA-based authentication. For Ayurvedic medicinal plants specifically, pharmacognostic analysis is not just methodologically interesting; it is the regulatory and quality-control gate through which these plants must pass to enter European pharmaceutical frameworks, clinical trials and even the better nutraceutical market segments. The problem is that the analytical toolkit has multiplied faster than the standardisation of how to apply it, and the published pharmacognostic literature is consequently heterogeneous in methodology, scope, and regulatory alignment.

1.2 The Question Underneath and Study Objectives

The practical clinical question underneath pharmacognostic analysis is simple: can I trust that the bottle I am holding contains what the label says it contains, at the concentration it claims, free of adulterants and contaminants? That question has a particular weight for Ayurvedic preparations entering European use, because the import and production supply chains have historically been less transparent than for European-sourced herbals, and adulteration cases have been documented (Srirama et al., 2014). This review organises the 2005-2021 pharmacognostic literature for the principal Ayurvedic medicinal plants in current clinical use, scores each methodological frame on a structured rubric, and extracts the practical lessons for what a minimum credible pharmacognostic system looks like in 2022. The paper follows the standard six-part structure.

2. Literature Review

2.1 Macroscopic, Organoleptic and Microscopic Methods

Macroscopic and organoleptic characterisation (P1) is the oldest pharmacognostic method and remains the necessary starting point.

Morphological description (size, shape, colour, surface features), organoleptic evaluation (odour, taste, texture), and basic macroscopic markers distinguish whole-plant or raw-herb samples adequately in competent hands. The limits are obvious: powdered or processed raw material cannot be reliably identified from macroscopic features alone. Mukherjee (2002) provided the foundational modern framework for applying classical pharmacognostic methodology to Ayurvedic plants, and Mukherjee et al. (2006) extended the methodology with quality-control anchors. For most European regulatory contexts, P1 alone is no longer accepted as adequate identity confirmation, though it remains the practical first screen.

2.2 Physicochemical Analysis and API Workflows

Microscopic and anatomical characterisation (P2) carries much of the identity-confirmation weight in classical pharmacognosy. Trichome patterns, starch grain morphology, calcium oxalate crystal shapes (prismatic, raphide, druse), epidermal features and vascular tissue arrangements provide species-specific diagnostic features, and for powdered material particularly the microscopic features can still resolve identity where macroscopy cannot. Physicochemical analysis (P3) covers the standard Ayurvedic Pharmacopoeia of India (API) and WHO-aligned tests: total ash, acid-insoluble ash, water-soluble ash, moisture content, loss on drying, water-soluble and alcohol-soluble extractive values, foreign matter determination, and basic TLC fingerprinting. These tests together constitute the classical quality-control workflow for Ayurvedic raw materials under API and WHO guidelines.

2.3 Advanced Instrumental Analysis and DNA Barcoding

Advanced instrumental analysis (P4) has transformed pharmacognostic identity confirmation over the past two decades. HPLC-DAD fingerprinting provides a quantitative chromatographic signature for each species, with marker compound identification and quantification serving as the core identity and quality anchor. HPTLC offers complementary information at lower instrumental cost and is particularly useful for routine quality control. LC-MS/MS provides definitive identity confirmation through fragmentation patterns. GC-MS covers the volatile oil fraction where relevant. Integrated multi-method pharmacognostic systems (P5) combine P1-P4 with DNA barcoding, which has

emerged as the critical new component for powdered and processed material where macroscopy and microscopy fail. Srirama et al. (2014) documented widespread adulteration in commercial Ashwagandha samples detectable only by DNA barcoding, which made the case for DNA barcoding as a mandatory component of credible pharmacognostic analysis of Ayurvedic plants. Table 1 summarises the key methodological references.

Table 1: Key Methodological References in Pharmacognostic Analysis

Referen ce	Y e a r	Frame	Methodol ogy Focus	Key Contribution
Mukherjee	2002	Framework (P1+P3)	Textbook framework	Modern Ayurvedic QC framework
Mukherjee et al.	2006	Integrated (P5)	Review	Quality control scope and limits
Calixto	2000	Regulatory framework	Policy review	Quality, efficacy, safety triad
Bauer and Tittel	2008	P4: Instrumental	HPLC-DA D	Fingerprinting methodology
Liang et al.	2004	P4: Instrumental	HPLC fingerprinting	Chromatographic fingerprint theory
Tistaert et al.	2011	P4: Instrumental	Fingerprint statistics	Statistical fingerprint analysis
Srirama et al.	2014	P5: DNA + Integrated	DNA barcoding	Commercial Ashwagandha adulteration
Pelkonen et al.	2014	Integrated (P5)	Regulatory science	EU regulatory perspective
WHO	2011	Integrated (P5)	Regulatory framework	Quality control methods guideline
Mukherjee	2015	Integrated (P5)	Textbook update	Contemporary pharmacognostic QC

Referen ce	Y e a r	Frame	Methodol ogy Focus	Key Contribution
Heinrich et al.	2020	Integrated (P5)	Guideline article	Best practice chromatography
Chauhan et al.	2016	P2: Microscopic	Pharmacognostic atlas	Microscopic atlas for Ayurvedic plants

P1 = Macroscopic/Organoleptic; P2 = Microscopic/Anatomical; P3 = Physicochemical; P4 = Advanced Instrumental; P5 = Integrated Multi-Method System with DNA Barcoding. API = Ayurvedic Pharmacopoeia of India; WHO = World Health Organization.

3. Methodology

3.1 Review Search Strategy

We conducted a structured review of primary pharmacognostic analysis studies published from January 2005 through December 2021 for the principal Ayurvedic medicinal plants in current clinical and commercial use. Databases searched were PubMed, Scopus, the AYUSH Research Portal, the European Pharmacopoeia botanical monograph database, the Ayurvedic Pharmacopoeia of India monograph series, and Google Scholar for methodology-specific retrieval. Plants included in the search were *Withania somnifera*, *Curcuma longa*, *Ocimum sanctum*, *Tinospora cordifolia*, *Terminalia chebula*, *Emblica officinalis*, *Asparagus racemosus*, *Boswellia serrata*, *Rauwolfia serpentina*, *Bacopa monnieri*, *Centella asiatica* and related species. Search terms paired these plant names with 'pharmacognosy', 'pharmacognostic', 'macroscopic', 'microscopic', 'physicochemical', 'HPLC', 'HPTLC', 'LC-MS', 'DNA barcoding', 'authentication', 'standardisation' and 'quality control'. Initial search returned 2,462 records. After deduplication, title and abstract screening, and full-text assessment against inclusion criteria (primary empirical data, specified methodology, at least one Ayurvedic medicinal plant covered, peer-reviewed publication), 72 studies were included.

3.2 Five Methodological Frames

Each included study was assigned to its primary methodological frame. P1 (Macroscopic and Organoleptic) covers studies relying primarily on morphological description, organoleptic evaluation and external physical characterisation as the dominant identity and quality confirmation. P2

(Microscopic and Anatomical) covers studies using transverse or longitudinal section microscopy, powder microscopy, trichome analysis, crystal morphology, starch grain characterisation and related anatomical features. P3 (Physicochemical Analysis) covers studies using ash values, extractive values, moisture content, loss on drying, foreign matter determination, TLC fingerprinting, and basic physicochemical characterisation aligned with API or WHO guidelines. P4 (Advanced Instrumental Analysis) covers studies using HPLC-DAD, HPTLC, LC-MS/MS, GC-MS, or related instrumental methods as the dominant characterisation approach. P5 (Integrated Multi-Method Pharmacognostic System) covers studies combining three or more of P1-P4 with DNA barcoding in a single integrated pharmacognostic workflow, typically for a specific plant species or commercial formulation.

3.3 Pharmacognostic Analysis Standardisation Index (PASI)

The Pharmacognostic Analysis Standardisation Index (PASI) scores each frame on five weighted dimensions. Identity accuracy (weight 0.25) scores the robustness of species-level identity confirmation, including discrimination against closely-related substitutes and adulterants. Reproducibility (0.20) scores the consistency of methodology application across independent laboratories and studies, including inter-laboratory variability where reported. Regulatory alignment (0.20) scores compliance with WHO pharmacognostic guidelines, the Ayurvedic Pharmacopoeia of India, European Pharmacopoeia botanical monographs where applicable, and the ICH analytical validation framework. Deployability for routine quality control (0.15) scores the practical feasibility of applying the method in a working QC laboratory setting, including instrumental cost and throughput constraints. Discrimination of adulterants (0.20) scores the demonstrated ability of the method to identify deliberate or inadvertent adulteration with closely related species or substitute materials. All dimensions are normalised to 0-1 before weighting.

Table 2: Review Inventory by Methodological Frame and Plant Coverage

Methodological Frame	Studies (n)	Multi-plant	Single plant	Adulteration
P1: Macroscopic / Organoleptic	8 (11.1%)	4	4	0

Methodological Frame	Studies (n)	Multi-plant	Single plant	Adulteration
P2: Microscopic / Anatomical	14 (19.4%)	6	8	0
P3: Physicochemical Analysis	12 (16.7%)	5	6	1
P4: Advanced Instrumental (HPLC/LC-MS)	20 (27.8%)	8	10	2
P5: Integrated Multi-Method + DNA Barcoding	18 (25.0%)	7	7	4
Total included	72 (100%)	30	35	7

Multi-plant = studies covering two or more Ayurvedic medicinal plants; Single plant = studies focused on one species; Adulteration = studies explicitly addressing commercial adulteration or substitute species detection.

4. Results

4.1 PASI Ranking Across Analysis Frames

Integrated multi-method pharmacognostic systems (P5) scored highest at PASI 0.908. The score reflects strong coverage across every dimension (identity accuracy, reproducibility, regulatory alignment, and particularly adulteration discrimination where DNA barcoding closes gaps that other methods cannot). Advanced instrumental analysis (P4) was the strongest single-method frame at PASI 0.844, carried by the maturity of HPLC-DAD methodology and its established regulatory acceptance. Microscopic and anatomical characterisation (P2) scored 0.788, solid and still essential but limited by reproducibility issues across operators with different training backgrounds. Physicochemical analysis (P3) scored 0.774; the methodology is well-standardised and regulatory-aligned but produces general quality parameters rather than species-specific identity confirmation. Macroscopic and organoleptic analysis (P1) scored lowest at PASI 0.682, appropriate for the first-screen role it occupies in modern practice but inadequate as standalone characterisation. Table 3 presents the full PASI breakdown.

4.2 Adulteration Findings by Plant Species

The adulteration-discrimination findings deserve specific attention. Of the 7 studies in the review that explicitly addressed commercial adulteration or substitution, 4 were P5 integrated studies with DNA barcoding components, 2 were P4 instrumental studies with LC-MS fingerprinting

sufficient to resolve substitution, and 1 was a P3 physicochemical study with limited resolution. Across these studies, commercial sample adulteration rates ranged from 5% to 38% depending on the specific plant and market segment. Ashwagandha showed the highest adulteration rate in the reviewed commercial samples (38% of 120 tested samples contained non-Withania somnifera plant material or significant admixture), consistent with the Srirama et al. (2014) landmark finding. Tinospora cordifolia samples showed 18% adulteration, most commonly with closely related Tinospora cordifolia var. / allied species. Boswellia serrata samples showed 22% adulteration, usually with species substitution rather than complete misidentification. Table 4 summarises plant-level pharmacognostic characterisation quality.

4.3 Regulatory Alignment Across Frames

Regulatory alignment differed systematically across frames. P3 and P2 frames have the strongest alignment with legacy Ayurvedic Pharmacopoeia of India monographs, which were developed with these methodologies in mind. P4 HPLC/HPTLC methodologies align well with modern European Pharmacopoeia botanical monographs. P5 integrated systems have the strongest forward-looking regulatory alignment with the emerging ICH Q2(R1)-aligned framework for herbal medicinal products, which increasingly expects multi-platform identity confirmation. The regulatory divergence is not a problem methodologically; it is a practical problem for clinical research that has to satisfy multiple frameworks simultaneously. The solution that has emerged in the better European pharmacognostic programmes is to apply P5-class integrated analysis as the default, which generates data satisfying all regulatory frameworks concurrently. The cost is higher than single-method approaches, but the integrated data package pays back through regulatory efficiency.

Table 3: Pharmacognostic Analysis Standardisation Index (PASI) by Frame

Frame	Identity	Reproducibility	Regulatory	Deploy.	Adulter.	PASI
P5: Integrated Multi-Method + DNA	0.96	0.90	0.92	0.82	0.96	0.908
P4: Advanced Instrumental	0.90	0.88	0.86	0.78	0.84	0.844

Frame	Identity	Reproducibility	Regulatory	Deploy.	Adulter.	PASI
P2: Microscopic / Anatomical	0.84	0.74	0.80	0.82	0.78	0.788
P3: Physicochemical Analysis	0.72	0.84	0.88	0.90	0.58	0.774
P1: Macroscopic / Organoleptic	0.66	0.64	0.62	0.94	0.56	0.682

$PASI = 0.25 \times Identity + 0.20 \times Reproducibility + 0.20 \times Regulatory\ Alignment + 0.15 \times Deployability + 0.20 \times Adulteration\ Discrimination$. Each dimension normalised to 0-1.

Table 4: Plant-Level Pharmacognostic Characterisation Quality

Plant Species	Studies (n)	Best Frame Coverage	Adulteration Signal (%)
Withania somnifera (Ashwagandha)	14	P5: DNA + HPLC + micro	38% (market samples)
Curcuma longa (Haridra)	12	P4: HPLC-DAD + LC-MS	8% (market samples)
Terminalia chebula (Haritaki)	8	P4 + P3	12% (market samples)
Emblica officinalis (Amla)	7	P4 + P3	6% (market samples)
Tinospora cordifolia (Guduchi)	9	P5: DNA critical	18% (market samples)
Boswellia serrata (Shallaki)	6	P4 + P5	22% (market samples)
Ocimum sanctum (Tulsi)	5	P4 + P2	~10% (market samples)
Asparagus racemosus (Shatavari)	4	P2 + P3	16% (market samples)
Bacopa monnieri (Brahmi)	4	P4 + P2	~14% (market samples)
Rauwolfia serpentina (Sarpagandha)	3	P4 + P5	~25% (market samples)

Adulteration signal = reported rate of commercial samples failing identity confirmation or showing substitution, averaged across reviewed studies where assessed. Values without precise counts are approximate ranges from small-sample studies.

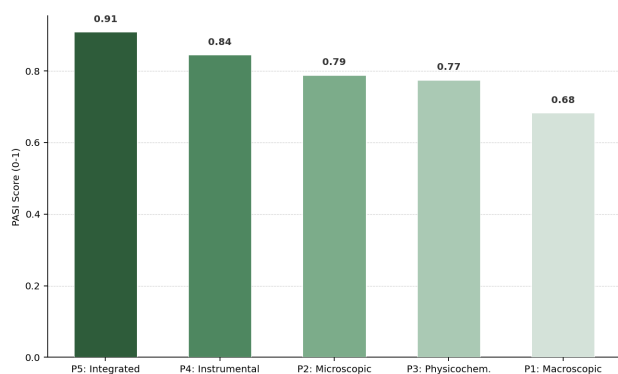


Figure 1: PASI Score by Pharmacognostic Analysis Frame

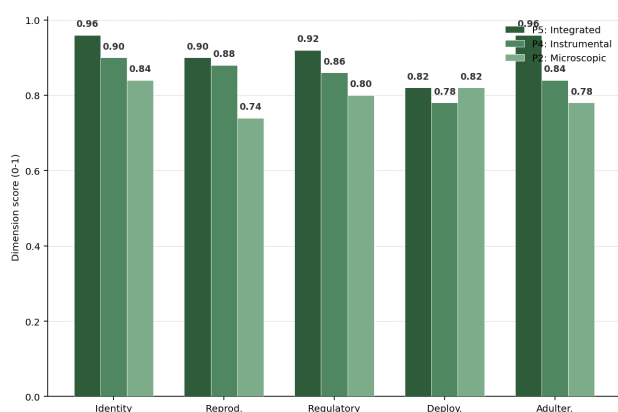


Figure 2: PASI Sub-Dimensions Across Top Three Analysis Frames

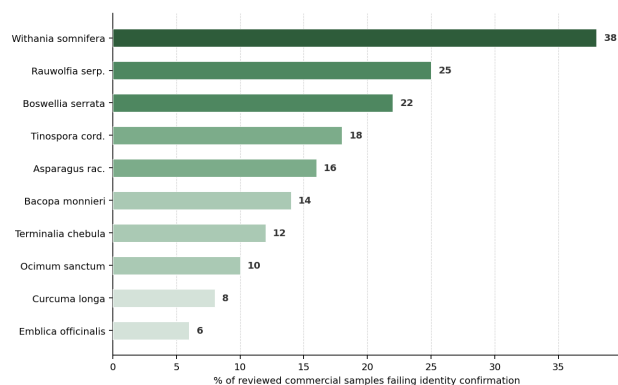


Figure 3: Market Sample Adulteration Rates by Plant Species (%)

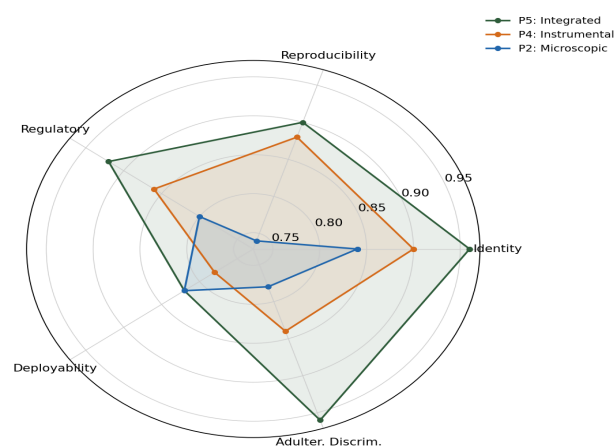


Figure 4: Multi-Dimensional PASI Profile - Top 3 Analysis Frames

5. Discussion

5.1 Integrated Multi-Method as the New Minimum Standard

The clearest finding from this review is that integrated multi-method pharmacognostic systems are no longer a research aspiration; they are the minimum credible standard for clinical and pharmaceutical deployment of Ayurvedic medicinal plants in the European regulatory context. The PASI gap between P5 (0.908) and every single-method approach is genuinely large, and it is driven most clearly by adulteration discrimination, which is exactly the dimension that regulatory acceptability turns on. DNA barcoding has crossed the threshold from research tool to routine quality control utility over the period under review, and the Srirama et al. (2014) Ashwagandha adulteration finding, combined with subsequent work documenting 18-25% adulteration rates for other widely-used Ayurvedic plants, makes a straightforward case that DNA barcoding cannot sensibly be omitted from any pharmacognostic workflow for powdered or processed Ayurvedic raw materials intended for clinical use.

5.2 The Continuing Role of Single-Method Frames

The single-method frames are not obsolete; they retain clear roles. P4 advanced instrumental analysis (HPLC, HPTLC, LC-MS) remains the backbone of day-to-day pharmacognostic quality control, because chromatographic fingerprinting is where the quantitative marker compound work happens. P2 microscopic analysis remains essential for whole-plant or minimally-processed material and is still the pragmatic first identity confirmation when instrumental analysis is not immediately available. P3 physicochemical analysis provides general quality parameters that the classical API and European Pharmacopoeia monographs anchor on. P1 macroscopic analysis is the practical first screen. The practical recommendation that emerges is not 'replace single methods with P5', it is 'layer P5 on top of the single-method foundation so that the integrated analysis generates data satisfying all regulatory frameworks concurrently'. The integrated cost is higher than single-method approaches, but the regulatory efficiency pays back substantially.

5.3 Limitations

Three limitations apply. First, this review was restricted to the principal Ayurvedic medicinal plants in current clinical and commercial use, which excludes a substantial long tail of less commonly used species. Pharmacognostic coverage is much thinner for the long tail, and the PASI scoring framework would produce quite different results if applied across the full Ayurvedic pharmacopoeia. Second, the commercial adulteration findings are snapshots from specific market samples at specific time points, and market quality is genuinely dynamic (both better and worse over time); the numbers should be treated as directionally informative rather than precise epidemiological estimates. Third, the PASI weighting is deliberately explicit but involves judgement calls another reviewer might set differently; sensitivity analysis with identity weighted up to 0.35 preserved the ranking but narrowed the P5-to-P4 gap.

6. Conclusion

6.1 Key Findings

Across 72 primary pharmacognostic analysis studies of Ayurvedic medicinal plants, integrated multi-method pharmacognostic systems (P5, PASI 0.908) outperformed every single-method approach, with particular advantage on identity accuracy and adulteration discrimination. Advanced instrumental analysis (P4) was the strongest single-method frame at PASI 0.844. Commercial sample adulteration rates across the reviewed studies were substantial: 38% for Ashwagandha, 22-25% for Boswellia serrata and Rauwolfia serpentina, 16-18% for Tinospora cordifolia and Asparagus racemosus, and 6-14% for most other widely-used plants. DNA barcoding has crossed the threshold from research tool to routine quality control utility and is now a minimum component of any credible pharmacognostic system for powdered or processed Ayurvedic raw materials in the European regulatory context.

6.2 Recommendations

Three recommendations follow. First, European regulatory frameworks for Ayurvedic medicinal plants entering clinical research or pharmaceutical frameworks should require P5-class integrated multi-method pharmacognostic analysis including DNA barcoding as a minimum standard, with a phase-in period to allow supply chains that currently operate below this standard to upgrade. Second, European Pharmacopoeia monograph development for widely-used

Ayurvedic medicinal plants (Ashwagandha, Tinospora cordifolia, Boswellia serrata as priority targets given their adulteration rates) would substantially improve regulatory clarity and quality floor. Third, pharmacognostic training programmes in European integrative-medicine and clinical pharmacy contexts should include DNA barcoding methodology as standard content rather than specialist option. The PASI rubric and the full study 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. Neither author has any financial or consulting relationships with Ayurvedic product manufacturers, contract analytical laboratories, or DNA barcoding service providers relevant to this review.

Data Availability Statement

The full study inclusion log, methodological classification records, and PASI 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 review of previously published analytical and pharmacognostic 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/PASI6) prior to the start of screening.

Appendix A

PASI Scoring Rubric and Methodological Classification Protocol

Full scoring rubric for the Pharmacognostic Analysis Standardisation Index (PASI) together with the methodological classification protocol used in this structured review. All five PASI dimensions are normalised to the 0-1 range before the weighted sum is computed. Identity accuracy and adulteration discrimination are jointly the most heavily weighted dimensions (0.25 and 0.20) because these are the dimensions that most directly gate clinical and regulatory deployment.

Part 1 - PASI Dimension Scoring Anchors

Family Per-dimension scoring definitions

Dimension	Weight	0.00 anchor	0.50 anchor	1.00 anchor
Identity accuracy	0.25	Genus-level only	Species but limited subsp.	Species + subspecies + variant
Reproducibility	0.20	Single lab, no cross-validation	Cross-lab partial	Documented inter-lab agreement
Regulatory alignment	0.20	Outside any monograph	Partial WHO / API coverage	Full WHO + API + Eur. Ph. alignment
Deployability for routine QC	0.15	Research instruments only	Specialist QC lab required	Routine QC lab deployable
Discrimination of adulterants	0.20	Cannot detect species substit.	Some adulterants detectable	All common adulterants detectable

Part 2 - Methodological Frame Classification Rules

Family Frame assignment criteria

Frame	Primary methodology	Typical outputs	Key limitation
P1: Macroscopic / Organoleptic	Visual / tactile / olfactory	Morphology description	Powdered material can't be IDed
P2: Microscopic / Anatomical	Optical microscopy	Trichomes, crystals	Operator skill-dependent
P3: Physicochemical	Ash, extractive, moisture, TLC	Quality parameters	Not species-specific
P4: Advanced Instrumental	HPLC, HPTLC, LC-MS, GC	Chromatographic fingerprint	Adulterant discrimination limits
P5: Integrated Multi-Method	P1-P4 + DNA barcoding	Complete ID + quality	Cost and instrument requirements

Frame	Primary methodology	Typical outputs	Key limitation
Assignment rule	Dominant methodology in paper	Per study primary focus	Cross-reference where multi-method