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STANDARDISATION OF TRIPHALA CHURNA USING GALLIC ACID AS AN ANALYTICAL MARKER

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Abstract

Triphala churna, a powder made from the dried fruits of three plants, is widely used in Ayurveda, yet its composition varies from batch to batch. This narrative review examines what the published literature says about standardising the formulation with gallic acid as an analytical marker. It covers the chemistry of the three fruits, pharmacopoeial parameters, extraction and chromatographic methods, validation, and contaminant testing. It reports no new data and describes no laboratory work on Triphala. The literature supports gallic acid as a practical marker that is measurable by HPLC or HPTLC, but also shows that extraction conditions strongly affect results and that a single marker cannot confirm botanical identity or clinical value.

1. INTRODUCTION

Triphala churna is among the most widely used polyherbal powders in Ayurveda. The name means three fruits, and the formulation combines the dried fruit pericarps of *Terminalia chebula* (haritaki), *Terminalia bellerica* (bibhitaki) and *Phyllanthus emblica* (amalaki), traditionally in equal parts [1, 2]. It is taken as a digestive and laxative, as a rejuvenating tonic, and for a long list of other complaints, and it is sold in India and abroad in several dosage forms [3, 4]. Because it is a mixture of three botanicals, each with its own chemistry and natural variability, two products sold under the same name can differ markedly in composition. That variability is the central problem for anyone who wants to

say what a given batch actually contains.

Standardisation is the answer proposed by regulators and pharmacognosists alike. In herbal medicine it means a set of tests that confirm identity, purity and, where possible, content of the constituents thought to matter, so that batches can be compared and held to a specification [5, 6]. For a single-plant drug, one chemical marker may do. For a three-fruit mixture the choice is harder, because no single compound is shared in equal measure by all three components. Gallic acid is the marker that comes closest, since it is present in all three fruits, either free or released from hydrolysable tannins, and it is easy to measure with common laboratory equipment [7, 8, 9].

This review examines what the published literature says about the standardisation of Triphala churna with gallic acid as an analytical marker. It covers the chemistry of the formulation, pharmacopoeial standards, extraction and analytical methods, method validation, contaminants and the limits of a single-marker approach. It reports no new data, and no experiments on Triphala are described. Findings are drawn from pharmacopoeial texts, regulatory guidance, phytochemical studies and pharmacological reports. Where a statement concerns Triphala itself it is attributed to a source, and where it concerns herbal quality control in general, that is made clear. The aim is to set out what a reasonable standardisation protocol would involve and to show where the evidence for it is incomplete.

A few words on terminology help. Standardisation in the herbal context is not the same as the standardisation of a synthetic drug, where purity can be defined absolutely. A standardised herbal extract is one adjusted to contain a defined amount of one or more markers, whereas standardisation of a crude powder like churna usually means establishing specification limits for a set of parameters. Active markers are compounds known to contribute to the activity, while analytical markers are chosen for convenience and do not necessarily carry the effect. Gallic acid in Triphala is best described as an analytical marker with some activity of its own [8, 10, 11].

2. CHEMISTRY OF TRIPHALA AND GALLIC ACID

The chemistry of the three fruits is dominated by polyphenols. *Terminalia chebula* and *T. bellerica* contain hydrolysable tannins such as chebulagic acid, chebulinic acid and corilagin, together with gallic acid and ellagic acid, whereas *P. emblica* is notable for its ascorbic acid content and for tannins that release gallic and ellagic acids on hydrolysis [9, 12]. Quantitative studies of Egyptian fruit samples reported large differences in polyphenol profile between species and in antioxidant capacity [9]. Seen from the formulator's side, the mixture is a complex system in which the same acids can appear free, bound or both, and the form in which they occur affects what a given extraction method yields [9, 13, 14].

Gallic acid (3,4,5-trihydroxybenzoic acid) is a small phenolic acid with a molecular mass of about 170 and strong absorption in the ultraviolet region, which makes it simple to detect by spectrophotometry and liquid chromatography. It is soluble in water and in polar organic solvents, and is relatively stable in acidic solution, though it oxidises in alkaline conditions and in the presence of light and air. These properties explain why acidified aqueous-methanolic solvents are so often used in its analysis [8, 13, 14]. Gallic acid is also a breakdown product of gallotannins, so heating, storage or extraction in water can increase the free acid content at the expense of tannins, a point that complicates comparisons between laboratories [9, 10].

The pharmacology of Triphala has been reviewed several times. Laboratory studies report free-radical scavenging by Triphala and each of its fruits [15], accelerated healing of infected dermal wounds in rats [16], cytotoxic effects against cancer cell lines and in animal models [17], and protection against gamma radiation in mice [18]. Clinical and traditional uses, in particular for functional gastrointestinal disorders, are discussed in reviews that also point to the shortage of well-controlled trials [3, 4, 19]. Gallic acid has its own reported antioxidant, anti-inflammatory and cytoprotective effects, and some authors argue that it contributes to

the activity of Triphala [8, 10]. Evidence that its content tracks clinical benefit in people is, however, lacking.

The relative contribution of each fruit to the final gallic acid content is not fixed. Because the three fruits differ in their tannin content, a 1:1:1 mixture by weight will not deliver equal amounts of gallic acid from each. Reports on Triphala sourced from different suppliers suggest that the proportion of components, plant part, geographical origin, harvest time and drying conditions all influence marker levels. A specification that fixes only the total gallic acid can therefore mask a change in the mixture, which is a reason to combine a marker assay with chromatographic fingerprinting [7, 11, 20].

The studies relevant to standardisation fall into four groups: biological activity that motivates quality control, chemical work that identifies markers, analytical methods that quantify them, and quality-control and contaminant studies that define acceptable limits. They are summarised in table 1 and counted by theme in fig. 1. The biological studies are the most numerous in the Triphala literature, whereas analytical work specific to the formulation is comparatively scarce, and often limited to a handful of markers [7, 15, 16, 17, 18]. That imbalance matters. A great deal has been written about what Triphala might do, and much less about how to make sure that each batch is the same product [7, 12, 21].

3. PHARMACOPOEIAL AND PHYSICOCHEMICAL STANDARDS

The starting point for any standardisation is the set of basic parameters in the pharmacopoeia and in the World Health Organization's methods for herbal materials. These include macroscopic and microscopic identity, foreign organic matter, moisture or loss on drying, total ash, acid-insoluble ash, and water-soluble and alcohol-soluble extractive values [11, 14]. Each parameter answers a different question. Ash values reflect inorganic content and adulteration with soil or sand, extractive values indicate the amount of material that dissolves in a solvent and so hint at quality and extraction efficiency, and moisture determines storage stability and microbial risk. The Ayurvedic Formulary of India describes the formula and general directions for preparing churna [2].

Official monographs for the single fruits provide limits that can be applied to the components before mixing, which is probably the most practical way to guard identity and purity [2]. Testing raw materials separately has an important advantage: adulteration or substitution can be detected early, and the proportions of the mixture can be controlled. Good practice also requires correct botanical authentication by a trained person, with reference specimens kept for comparison. Work on bringing medicinal plants into cultivation stresses that quality begins in the field, with correct species, appropriate harvest time and careful drying, and not only in the laboratory [22].

Table 1: Published findings relevant to standardisation of Triphala churna

Topic or method	Principal finding reported	Ref.
Antioxidant activity Triphala and its constituents	Free-radical scavenging demonstrated in vitro	[15]
Infected dermal wound rat model	Triphala promoted wound healing	[16]
Cancer cell lines and animals	Cytotoxic effects reported for Triphala	[17]
Gamma radiation mouse model	Protective effect against radiation damage	[18]
Reversed-phase HPLC	Several marker polyphenols including gallic acid quantified in Triphala	[7]
Folin–Ciocalteu assay	Total phenols expressed as gallic acid equivalents; not specific	[23]
HPTLC fingerprinting	Many samples compared on one plate with quantification	[24, 25]
Terminalia fruit polyphenols	Gallic acid and related compounds quantified in fruits	[9]
Pharmacopoeial parameters ash, extractives, moisture	Basic identity and purity tests set limits for the powder	[11, 14]
Metals in Ayurvedic products	Detectable lead, mercury or arsenic in a proportion of products	[26, 27]

Macroscopic and microscopic examination of powdered drugs remains useful and cheap. Characteristic cells, fibres, crystals and stone

cells identify each fruit in the powder, and a trained analyst can often detect gross adulteration or an altered ratio [5, 11, 14]. Phytochemical screening by simple colour reactions, for example with ferric chloride for phenolics, supports identity but is not specific. Such methods set the stage for chromatographic fingerprinting, which gives a pattern of bands or peaks characteristic of the whole formulation. The pharmacopoeial approach, then, builds up identity and purity from simple, inexpensive tests, and adds marker quantification as a measure of strength [11, 14, 24].

Storage and packaging affect the same parameters. Powdered fruit pericarp absorbs moisture readily, and rising water content encourages mould growth and speeds the hydrolysis of tannins, which in turn alters free gallic acid. Airtight, light-resistant containers and low humidity are the usual recommendations for herbal powders, and shelf life should be supported by stability data and not assumed from tradition [11, 28]. For a polyherbal powder, retained samples from each batch let a laboratory investigate complaints later. These measures are unglamorous, but they protect the validity of every later assay and cost little compared with the cost of discarding a batch [5, 22, 28].

4. EXTRACTION AND ANALYSIS OF GALLIC ACID

Extraction is the first source of variation in the assay. Solvents used for polyphenols include water, methanol, ethanol and their mixtures with water, often acidified to improve stability. The method (maceration, reflux, Soxhlet, ultrasound or microwave assistance), the particle size, the solid-to-solvent ratio and the time and temperature all influence yield [29, 30]. For a hydrolysable-tannin-rich material, prolonged heating in water can raise the free gallic acid by hydrolysis, which could be mistaken for a richer product. A protocol intended for quality control should therefore fix the extraction conditions precisely and use the same ones every time [29, 30].

Chromatographic methods are preferred for specific measurement of gallic acid. A published reversed-phase HPLC method quantified several marker polyphenols, including gallic acid, in Triphala and in its three components, and proposed the approach for routine standardisation [7]. Typical conditions use a C18 column with a gradient of acidified water and methanol or acetonitrile and ultraviolet detection around 270 nm. High-performance thin-layer chromatography is a cheaper alternative with the advantage that many samples can be run at once on one plate, and it provides a visual fingerprint as well as a quantitative result [24, 25]. Both methods separate gallic acid from other phenolics, which a simple colorimetric assay cannot do.

The Folin–Ciocalteu assay measures total phenolics, conventionally expressed as gallic acid equivalents [23]. The result is often reported in Triphala papers, and it is a useful screening measure, but it responds to any reducing substance, including ascorbic acid, which is abundant in amla. A high value in a mixture containing amla may therefore reflect vitamin C rather than polyphenols. For this reason, a figure of gallic acid equivalents should not be confused with a measured gallic acid content, and the two terms should not be used interchangeably in a specification [7, 23]. Chromatographic assay is required whenever the claim is about gallic acid itself.

The practical detail of HPTLC deserves comment, since many laboratories in India rely on it. Extracts are applied as bands on silica plates, developed in a solvent system suited to phenolic acids (commonly toluene, ethyl acetate and formic acid in varying proportions), and scanned by densitometry at a wavelength near the absorption maximum of gallic acid. Identity is supported by matching Rf value and spectrum with the reference standard, and plates can be derivatised with a spray reagent for visual comparison [24, 25]. The method is rapid and economical, but its precision is generally lower than that of HPLC, and plate-to-plate variation requires that standards be run on every plate [7, 25].

Sample preparation also matters at the stage of quantification. Standard solutions are prepared from authentic gallic acid, calibration is done over a defined range, and system suitability parameters such as

retention time, tailing and plate count are checked. Powdered churna is hygroscopic and inhomogeneous, so a representative sample needs adequate mixing and the result is best expressed on a dried basis. Because the assay result is only as dependable as the sampling, the sampling procedure should be written down in the same detail as the instrument conditions [5, 7, 25, 31].

Alternatives to chromatography have been explored. Ultraviolet spectrophotometry after separation, capillary electrophoresis and spectroscopic fingerprinting with chemometrics have each been proposed for herbal quality control. They may offer speed or lower solvent consumption, but they tend to need more elaborate validation to prove selectivity in a complex matrix. Published comparisons suggest that HPLC remains the reference technique for a marker such as gallic acid, while rapid methods are better thought of as screening tools. Their appeal is greatest where laboratories lack expensive equipment, as small manufacturers often do [6, 20, 25].

5. METHOD VALIDATION AND QUALITY ASSURANCE

An analytical method for a marker compound is only trustworthy if it has been validated. Validation guidance, notably the International Council for Harmonisation guideline Q2(R1) and its pharmacopoeial counterparts, lists the characteristics to be demonstrated: specificity, linearity, range, accuracy, precision (repeatability and intermediate precision), detection limit, quantitation limit and robustness [31]. For gallic acid in Triphala, specificity means showing that the peak is pure and is not overlapped by other phenolics, which can be checked by spiking and by diode-array spectra. Linearity is shown over a range bracketing the expected content, and accuracy is usually assessed by recovery after adding known amounts of the standard to the sample [7, 31].

Quality assurance extends beyond a single assay. The World Health Organization's main principles of good manufacturing practice call for documented specifications for starting materials and finished product, written procedures, batch records and retention of samples [28]. Stability should also be assessed, since gallic acid content may change on storage through oxidation or hydrolysis of tannins. Review articles on the standardisation of herbal drugs make the point that validated markers, reference standards and fingerprints must be used together, because a single number rarely captures the quality of a complex mixture [6, 20]. Acceptance limits are best set from data across many batches and not from a single laboratory run [6, 11].

Robustness is the characteristic most often skipped in published herbal assays. It tests whether small, deliberate changes in conditions, such as mobile phase pH, column temperature, flow rate or extraction time, leave the result unchanged. In a gallic acid assay, a change in solvent acidity can shift retention and the apparent concentration, so the allowed tolerances should be stated. System suitability checks run at the start of each analytical session then show that the instrument is performing as it did during validation [31]. Without such information a method may work well for its author and fail in a second laboratory, which defeats the purpose of standardisation [7, 25, 31].

Reference standards and reference materials deserve more attention than they get. Authentic gallic acid is easy to purchase, but a well-characterised reference batch of Triphala powder, against which laboratories could compare their results, is not generally available. Without one, an interlaboratory difference of ten or twenty per cent cannot be traced to its cause. Authors in the field have repeatedly called for such materials, and for collaborative trials to establish the precision of methods between laboratories rather than within one. Pharmacopoeial commissions are the natural bodies to organise such work [6, 11, 25].

A final quality question concerns reporting. Papers on herbal analysis often state a percentage of gallic acid without giving the basis (dry weight or as-received), the extraction details or the number of replicates. Such omissions make comparison impossible, even when the analytical work was done well. Journals and regulators increasingly ask for these details, and guidance on herbal quality

repeats the request for transparent methods and reference materials [5, 6, 20]. A reader should be able to repeat the calculation from the raw peak areas, state the dilution factors used, and see how many independent preparations of the powder were analysed. Where only a single extraction was run, the reported figure carries little weight, however many decimal places it is given to.

6. CONTAMINANTS AND SAFETY

Purity is as important as content. Contamination is a recurring theme in the herbal literature, and the usual concerns are microbial load, heavy metals, pesticide residues and mycotoxins [32, 33]. Plant materials are exposed to soil, water, handling and storage, and a powdered mixture offers ample opportunity for microbial growth when moisture is high. Testing for total viable count and specified pathogens is therefore a routine requirement. Cultivation in soils containing heavy metals can lead to accumulation in the plant, and a review of toxic contaminants in herbal medicines discusses the main sources and the limits that have been proposed [33].

Concern about metals in Ayurvedic products is well documented. A survey of Ayurvedic medicines purchased in Boston-area stores found detectable lead, mercury or arsenic in a proportion of the products tested [26], and an earlier review of traditional Indian remedies had likewise reported toxic metals in some of the products examined [27]. These reports mostly concerned preparations in which metals are an intentional ingredient or products of uncertain provenance, and they should not be read as showing that plain herbal powders such as Triphala are contaminated. They nonetheless explain why regulators ask for heavy metal testing of all Ayurvedic products, and why a complete specification for churna includes it [26, 27, 32].

Safety also involves the plant material itself. Triphala has a long history of use and is generally regarded as well tolerated, with loose stools and abdominal discomfort the effects most often noted, but systematic safety data from long-term trials are limited [3, 4, 19]. Adulteration with unrelated fruits or with synthetic laxatives or dyes is a theoretical concern for any churna sold without batch testing. Pharmacists asked about herbal products should be aware that the label of an Ayurvedic proprietary product may not state the proportion of its components, that regulatory oversight differs between countries, and that quality can vary widely between brands [21, 33].

7. LIMITATIONS AND FUTURE DIRECTIONS

Using gallic acid as the sole marker has limits. It is present in many plants and in many other herbal products, so it says little about botanical identity, and it can be generated during processing. A batch could meet a gallic acid specification and still contain the wrong ratio of fruits or an inferior grade of one of them. For these reasons, specialists recommend using gallic acid alongside other markers, such as ellagic acid and chebulagic acid, and chromatographic fingerprints that reflect the whole profile [6, 7, 9, 11]. The pharmacological studies cited in support of Triphala also come mainly from preclinical models, and their relevance to human use is unproven [15, 16, 17, 18].

Future work would benefit from several steps. Multi-laboratory validation of a gallic acid assay for Triphala would show whether results are reproducible between sites. Specification limits should be based on data from many commercial batches, with attention to region and season [11, 20]. Links between marker content and clinical effect need testing in controlled studies, which are few [3, 19]. Together with improved reference materials and agreement on extraction conditions, these measures would turn current practice from a collection of local methods into a shared standard [6, 21, 31]. Until then, results reported for gallic acid in Triphala churna should be read with the method in view. Manufacturers, too, have a part to play by publishing their specifications and accepting external audit of their batch records [21, 28].

8. CONCLUSION

Published work supports gallic acid as a practical analytical marker for Triphala churna. It occurs in all three fruits, is easy to

detect, and can be measured specifically by liquid or thin-layer chromatography [7, 8, 9, 24]. Its assay sits best within a wider scheme that also checks identity, ash, extractive values, moisture and contaminants according to pharmacopoeial and published quality-control methods [5, 14, 32]. Method validation to the ICH standard is what gives the number its meaning [31].

At the same time, a gallic acid figure on its own is a weak guarantee. Extraction conditions can alter the free acid content, colorimetric assays overstate it, and a single marker cannot show that the three fruits are present in the right proportion or that the product works [6, 11, 23]. Better reference materials, multi-laboratory validation and limits drawn from many batches are the steps most likely to improve practice [20, 25]. Until then, reports of gallic acid in Triphala are best read together with the method that produced them.

9. ABBREVIATIONS

HPLC-High-performance liquid chromatography, HPTLC-High-performance thin-layer chromatography, ICH-International Council for Harmonisation, WHO-World Health Organization, GMP-Good manufacturing practice, UV-Ultraviolet

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11. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

12. CONFLICT OF INTERESTS

Declared none

13. REFERENCES

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