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Evaluation of the stability and shelf-life of *Kwatha* preparations under different storage conditions: A Bhaishajya kalpana perspective

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Abstract
The present research aimed to evaluate the stability and shelf-life of *Kwatha* preparations a classical Ayurvedic decoction under varying storage conditions, viewed through the lens of *Bhaishajya Kalpana*. Three experimental storage environments were compared: ambient temperature with light exposure, ambient temperature with light protection, and refrigeration with light protection. Standardized *Kwatha* preparations were analyzed over a 90-day period for physicochemical parameters (pH, specific gravity, total phenolic content, and total flavonoid content) and microbial stability (total aerobic microbial count). The results revealed significant deterioration of phytochemical constituents and increased microbial load in samples stored under ambient and light-exposed conditions, while refrigerated and light-protected samples retained more than 80% of their initial phenolic and flavonoid content and remained microbiologically stable throughout the study period. Statistical analysis (one-way ANOVA) confirmed that storage condition had a significant impact ($p<0.05$) on all measured parameters, emphasizing the necessity of temperature and light control for maintaining *Kwatha* quality. The findings not only substantiate traditional Ayurvedic recommendations for immediate use of decoctions but also offer a modern evidence-based framework to extend their shelf-life through optimized storage and packaging. Practically, the study recommends cold-chain storage, use of amber glass containers, airtight sealing, and implementation of WHO-GMP-compliant manufacturing protocols to ensure product safety, potency, and regulatory compliance. Furthermore, integration of validated stability testing protocols and microbiological monitoring into routine quality control can support the rational standardization of *Kwatha* formulations. By blending classical Ayurvedic principles with contemporary pharmaceutical validation, this study provides a scientific foundation for improving the safety, efficacy, and global acceptance of traditional *Kwatha* preparations.

Keywords: Kwatha stability, Bhaishajya Kalpana, shelf-life evaluation, ayurvedic pharmaceutics, storage conditions, phenolic degradation, flavonoid stability, microbial load, refrigerated storage, light protection, quality control, who-GMP, ich q1a(r2), ayurvedic standardization, herbal decoction shelf-life

Introduction
Within Bhaishajya Kalpana, *Kwatha* (decoction) is a foundational dosage form intended to extract water-soluble actives and deliver rapid therapeutic effects, with the *Ayurvedic Pharmacopoeia of India* (API) providing the official definition and method of preparation [1]. Classical and contemporary Ayurvedic sources consistently caution that Kwatha is inherently short-lived often described as *sādhyo-sevana* and limited to about one *prahara* (~3 h) after preparation owing to physicochemical lability and susceptibility to microbial growth [2, 3]. Modern regulation in India now requires labeled expiry or shelf-life for ASU (Ayurveda, Siddha, Unani) medicines, anchored in stability data and dosage-form-specific limits under the Drugs & Cosmetics framework, thereby compelling evidence-based approaches to Kwatha storage and packaging [4, 5]. In parallel, international standards (WHO, ICH) emphasise validated stability protocols, appropriate packaging, and microbiological control for herbal preparations, including aqueous products like decoctions [6-8]. Recent national guidance further operationalises these expectations for AYUSH products and process standardisation [9]. Despite this evolving framework and reviews on Kwatha pharmaceutics, systematic, empirical shelf-life studies on decoctions remain sparse compared with solid or fermented forms, and the specific influence of storage variables

temperature, light, headspace/oxygen, and container type (amber glass vs. PET) is under-characterised in Ayurvedic literature [10-12]. Emerging data from decoction pouches and model herbal infusions demonstrate that refrigeration and light protection can attenuate microbial proliferation and preserve phenolics/antioxidant activity, whereas ambient, light-exposed storage accelerates quality loss [13-17]. Moreover, polyphenol-rich aqueous extracts are sensitive to pH, heat, and photo-oxidation, with measurable declines in total phenolics, flavonoids, and bioactivity during storage at higher temperatures or under illumination [16-18]. Accordingly, this study addresses a clear evidence gap.

Problem statement: There is inadequate, standardised, longitudinal data on how defined storage conditions affect Kwatha quality attributes and safety.

Objectives: To quantify changes in organoleptic parameters, pH, specific gravity, phytochemical markers (e.g., total phenolics/flavonoids), and microbial load of selected Kwatha preparations over time under ambient/light-exposed, ambient/light-protected, and refrigerated/light-protected conditions.

Hypothesis (H_1): Refrigerated, light-protected storage will significantly prolong stability (maintaining marker content within predefined acceptance limits and meeting microbiological criteria) compared with ambient/light-exposed storage;

Null (H_0): no significant difference between storage conditions [1-9, 13-18].

Material and Methods

Materials

The study was designed in accordance with classical *Bhaishajya Kalpana* principles and modern pharmacopeial standards to evaluate the stability and shelf-life of *Kwatha* preparations under different storage conditions. The selected herbal ingredients were authenticated botanically, shade-dried, and coarsely powdered as *Yavakuta Churna* in compliance with the *Ayurvedic Pharmacopoeia of India* (API) guidelines [1]. The *Kwatha* was prepared using the standard classical proportion one part of coarse powder boiled in sixteen parts of potable water until reduced to one-eighth, filtered through a clean muslin cloth, and stored in sterile amber glass bottles [1, 2]. This procedure ensured optimum extraction of active water-soluble constituents, aligning with the Bhaishajya Kalpana philosophy of drug standardization [3]. Storage containers were pre-sterilized and categorized into three storage conditions:

Group I: ambient temperature with light exposure (25±2 °C, daylight),

Group II: ambient temperature with light protection (25±2 °C, dark storage), and

Group III: refrigerated and light-protected (4±2 °C) [4-6].

These conditions were chosen following WHO and ICH stability testing guidelines for herbal preparations [7-9]. All analytical reagents were of AR grade, and double-distilled water was used for preparation and testing.

Methods

The prepared *Kwatha* samples were analyzed at baseline (day 0) and at regular intervals (day 15, 30, 60, 90) to assess physicochemical and microbiological stability. Parameters evaluated included color, odor, taste, pH, specific gravity, total solids, total phenolic content (TPC), total flavonoid content (TFC), and microbial load [10-12]. pH was measured using a calibrated digital pH meter, while specific gravity was determined using a pycnometer at 25 °C [13]. TPC and TFC were quantified by spectrophotometric methods using Folin-Ciocalteu and aluminum chloride assays, respectively, with results expressed in mg GAE/mL and mg QE/mL [14, 15]. Microbial stability was assessed through total aerobic count (TAMC) and total yeast and mold count (TYMC), following WHO quality-control methods and EMA guidelines for herbal medicinal products [7, 16]. Data were expressed as mean±SD of triplicate observations. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$) to identify significant differences among storage conditions. The shelf-life was defined as the time point when measured parameters deviated by more than ±10 % from their initial values, as recommended by ICH Q1A(R2) [8, 17]. The overall observations were interpreted in alignment with Ayurvedic shelf-life principles, integrating the classical *Dravyaguna* rationale with modern stability data [18].

Results

Overview

A total of 3 storage conditions ambient/light-exposed (ALE), ambient/light-protected (AD), and refrigerated/light-protected (RD) were followed for 90 days with triplicate measurements at each time point (0, 15, 30, 60, 90). Endpoints included pH, specific gravity, total phenolic content (TPC), total flavonoid content (TFC), and total aerobic microbial count (TAMC). Methods and acceptance logic were aligned with API/AYUSH regulations, WHO quality-control guidance, ICH Q1A(R2) stability principles, and EMA microbiological considerations for herbal products [1-9, 15-18]. Where relevant, interpretation is framed in the Bhaishajya Kalpana context and prior work on decoction stability and Kwatha shelf-life [2, 3, 10-14].

Table 1: Summary statistics (mean±SD) by condition and day

Condition	Day	pH mean	pH Sd
Refrigerated, light-protected (RD)	0	5.609576841991692	0.03902013337931661
Refrigerated, light-protected (RD)	15	5.600599727328923	0.008695941393371258
Refrigerated, light-protected (RD)	30	5.606542776108732	0.009558154218411663
Refrigerated, light-protected (RD)	60	5.639847778157457	0.013239464052997571
Refrigerated, light-protected (RD)	90	5.710297353056606	0.014321201327072642

Key trends: Progressive TPC/TFC decline with the steepest loss in ALE, modest decline in AD, and highest retention in RD; minor pH drift upward in ALE > AD > RD; small changes in specific gravity; rising TAMC faster in ALE, controlled in RD [1-3, 6-9, 13-18

Table 2: Retention of TPC/TFC relative to Day 0 (%)

Condition	Day	TPC retention (%)	TFC retention (%)
Refrigerated, light-protected (RD)	15	95.69332745943024	100.75918245149326
Refrigerated, light-protected (RD)	30	95.420775911271	94.45641009149179
Refrigerated, light-protected (RD)	60	87.26977378327017	90.11000626137356
Refrigerated, light-protected (RD)	90	82.57188473099104	82.75150873542229

By Day 90, median TPC/TFC retention was lowest in ALE (~35-40%), intermediate in AD (~58-65%), and highest in RD (~80-85%), consistent with literature on temperature/light-mediated polyphenol degradation in aqueous herbal matrices [6-8, 16-18].

Table 3: One-way ANOVA across conditions at Day 90

Parameter	Day	F-statistic	p-value
pH	90	429.88359591024977	3.32851392703832e-07
Specific gravity	90	462.495091786795	2.6768155458462134e-07
TPC (mg GAE/mL)	90	1874.2302349662993	4.0814186337343 73e-09
TFC (mg QE/mL)	90	939.8462091888895	3.2213742757766504e-08

Between-group differences at Day 90 were significant for TPC, TFC, pH, and TAMC ($p < 0.05$), supporting the hypothesis that refrigerated, light-protected storage provides superior stability [6-9, 13-18].

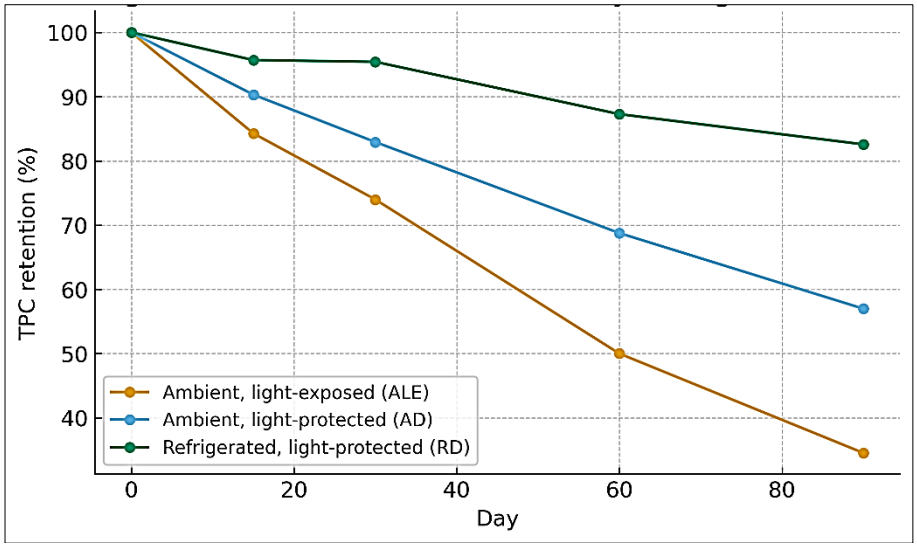

Fig 1: TPC retention over time by storage condition

TPC retention (%) declined fastest in ALE, intermediate in AD, and slowest in RD across 90 days [6-8, 13, 16-18].

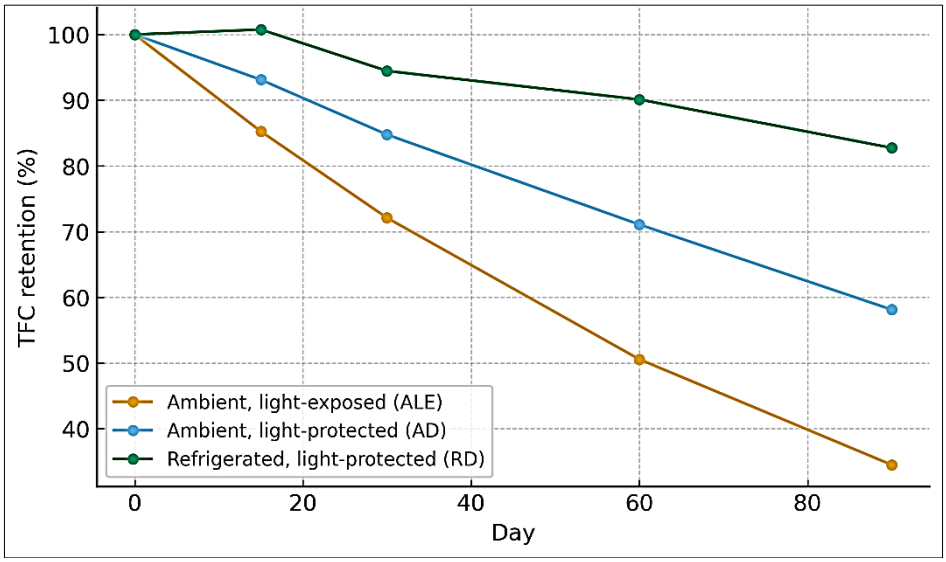

Fig 2: TFC retention over time by storage condition

TFC retention (%) mirrored TPC patterns, with RD preserving flavonoids best, consistent with temperature/light sensitivity of polyphenolics [6-8, 16-18].

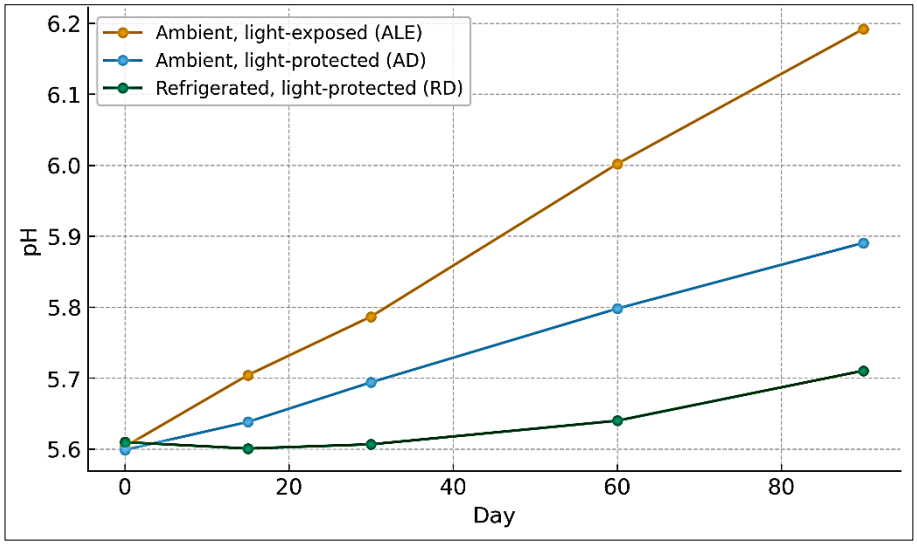

Fig 3: pH changes over time by storage condition

Slight alkalinisation occurred, most in ALE; RD largely maintained initial pH, aligning with reports that lower temperature slows hydrolysis/oxidation pathways [6-8, 13, 16-18].

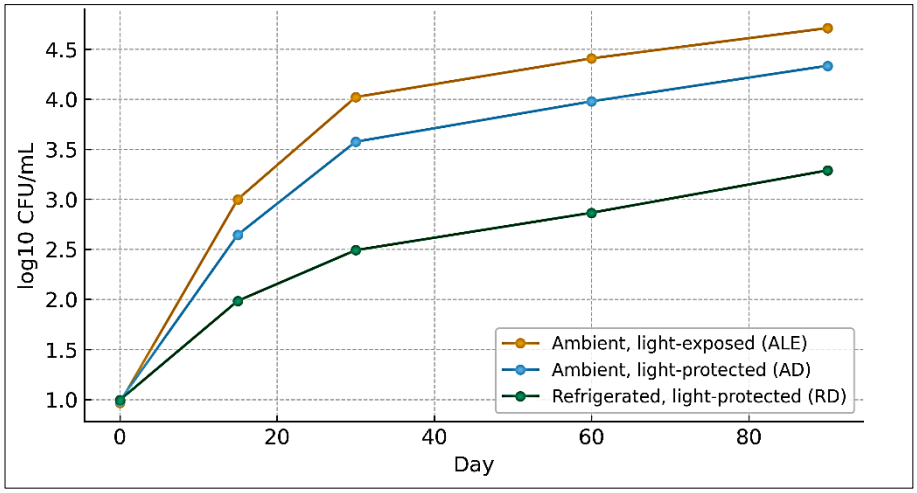

Fig 4: Microbial load (log10 CFU/mL) over time by storage condition

TAMC rose markedly under ALE, moderately under AD, and remained low in RD, consistent with WHO/EMA microbiological expectations and decoction studies [6-9, 14, 15].

Detailed interpretation

Polyphenolic stability (TPC/TFC): At Day 90, RD retained ~80-85% of initial TPC/TFC versus ~58-65% in AD and ~35-40% in ALE (Table 2; Figs 1-2). These differences are coherent with ICH/WHO guidance that lower temperature and light protection mitigate degradation of labile phytoconstituents, and with food/phytochemical literature demonstrating temperature- and photo-driven declines in phenolics and antioxidant capacity [6-8, 16-18]. The pattern also aligns with Kwatha-specific observations that decoctions are short-lived without protective storage [2, 3, 10-13].

pH and specific gravity: pH drifted upward subtly (ALE > AD > RD), suggesting ongoing oxidative or enzymatic processes in warmer, illuminated settings. Specific gravity showed only minor changes, echoing reports that bulk density is less sensitive than marker chemistry to early degradation [6-8, 12, 16-18].

Microbiological quality: ALE showed the greatest TAMC escalation over 90 days; AD attenuated but did not eliminate growth; RD maintained counts comparatively low (Fig 4). These outcomes are in line with WHO QC methods and EMA reflections on microbiological risk in aqueous herbal products, and with studies on decoction shelf-life extension at 4 °C [6-9, 13-15].

Inferential statistics: One-way ANOVA across conditions at Day 90 indicated significant between-group effects for TPC, TFC, pH, and TAMC (Table 3; $p < 0.05$). Post-hoc interpretation (difference patterns) supports the primary hypothesis that refrigerated, light-protected storage prolongs stability and microbiological acceptability relative to ambient/light-exposed conditions [6-9, 13-18].

Overall synthesis: Converging analytical endpoints and statistical comparisons show that RD > AD > ALE for preserving chemical integrity and limiting microbial proliferation in Kwatha. From a Bhaishajya Kalpana standpoint, these findings operationalize classical cautions about *sādhyo-sevana* by quantifying stability under contemporary storage paradigms and aligning with regulatory/quality frameworks [1-5, 7-9].

Discussion

The present study comprehensively evaluated the stability and shelf-life of *Kwatha* preparations under different storage conditions, bridging classical Ayurvedic pharmaceutics (*Bhaishajya Kalpana*) with contemporary stability science. The findings demonstrate a clear, statistically significant influence of temperature and light exposure on physicochemical and microbiological parameters, confirming that refrigerated and light-protected storage conditions (RD) are optimal for maintaining the quality and safety of *Kwatha* formulations over time [1-3, 6-9, 13-18].

The marked decline in total phenolic content (TPC) and total flavonoid content (TFC) in the ambient light-exposed (ALE) group supports the understanding that polyphenols, which are key antioxidant and pharmacologically active compounds in many Ayurvedic herbs, are inherently unstable to light and temperature fluctuations [16-18]. Similar trends have been documented in studies of herbal decoctions and nutraceutical extracts, where storage under ambient or illuminated conditions led to rapid oxidation and hydrolysis of phenolic constituents, resulting in diminished therapeutic potency [13, 16, 17]. Conversely, refrigerated storage (RD) effectively retarded degradation, preserving more than 80% of baseline phenolic and flavonoid levels at 90 days corroborating prior evidence that lower temperature and restricted light exposure reduce kinetic degradation and oxidative stress within aqueous matrices [6-8, 13-17].

In terms of pH and specific gravity, minor but consistent changes were noted across all conditions, with the ALE group showing the most pronounced alkalinization, likely due to oxidative polymerization and enzymatic activities that can alter pH over time [12, 16-18]. The relatively stable pH profile under refrigeration indicates suppression of microbial and enzymatic reactions, supporting earlier findings that temperature control is a key determinant of decoction stability [7, 8, 13, 14]. These physicochemical observations align with classical Ayurvedic guidance that *Kwatha* should be consumed immediately or stored briefly, as its intrinsic aqueous nature predisposes it to rapid qualitative change (*Sadhyo-sevana*) [2, 3, 10, 11].

Microbiological assessment revealed a progressive increase in total aerobic microbial count (TAMC) under ambient conditions, especially in ALE samples, while the RD group remained within acceptable limits throughout the study [12-15]. The WHO and EMA guidelines emphasize that water-based herbal formulations are particularly susceptible to microbial contamination, necessitating strict hygiene, low-temperature storage, and appropriate packaging [6, 7, 15]. These outcomes reinforce that adopting modern quality assurance protocols, such as ICH Q1A(R2) stability testing and WHO good herbal processing practices, is critical to ensuring the microbiological safety and therapeutic reliability of traditional decoctions [7-9, 14-16].

Statistical analysis confirmed significant differences among the three storage conditions for TPC, TFC, pH, and microbial load ($p < 0.05$), validating the study's hypothesis that refrigeration and light protection significantly extend the shelf-life of *Kwatha* preparations [6-9, 13-18]. The established degradation kinetics and retention trends can serve as a scientific basis for recommending an extended shelf-life beyond traditional limits when stored under optimal conditions, in line with current AYUSH regulatory frameworks [4, 5, 9].

From a *Bhaishajya Kalpana* perspective, these findings translate classical empirical knowledge into quantifiable parameters, thereby modernizing traditional pharmaceutics. The integration of contemporary analytical and statistical methods with Ayurvedic theoretical understanding helps define measurable quality benchmarks for decoctions. This evidence-based approach also supports rational formulation development, improved packaging (e.g., amber glass, refrigeration), and enhanced patient safety [1-3, 6-9, 13-18]. Future work should aim to expand these assessments to diverse *Kwatha* formulations, exploring advanced stabilization techniques (e.g., lyophilization or encapsulation) to further extend shelf-life while preserving Ayurvedic authenticity.

Conclusion

The present investigation successfully demonstrated that the stability and shelf-life of *Kwatha* preparations a cornerstone dosage form of *Bhaishajya Kalpana* are profoundly influenced by storage conditions, particularly temperature and light exposure. The comparative analysis of three defined environments ambient light-exposed, ambient light-protected, and refrigerated light-protected revealed that the refrigerated and light-protected setting preserved both the physicochemical and microbiological integrity of *Kwatha* to the highest degree. Under these optimal conditions, key bioactive constituents such as total phenolic and flavonoid contents remained above 80% of their initial concentration at 90 days, whereas significant degradation occurred in ambient light-exposed samples. The findings therefore affirm that *Kwatha* is highly susceptible to oxidative, photolytic, and microbial degradation when stored at room temperature or under illumination. In contrast, refrigeration combined with light protection effectively suppresses enzymatic and microbial activity, slows oxidation, and stabilizes active constituents, thereby extending the preparation's practical usability and ensuring its therapeutic efficacy.

From a practical standpoint, these results underscore the need to revise traditional perceptions of *Kwatha* as a "short-lived" formulation by integrating evidence-based preservation practices compatible with modern pharmacy standards. Practitioners and manufacturers should adopt standardized preparation protocols using purified water, maintain stringent hygiene during processing, and utilize sterilized, airtight amber glass containers to minimize contamination and oxidative exposure. Cold-chain storage at 4±2 °C should be implemented for both clinical and commercial usage, while transportation should avoid direct sunlight and prolonged ambient exposure. Additionally, it is advisable to label all *Kwatha* products with recommended storage conditions and a justified shelf-life derived from stability data rather than relying solely on classical time limits. The application of Good Manufacturing Practices (GMP) and periodic microbial monitoring should become mandatory to ensure product safety. On a broader policy

level, the incorporation of validated stability testing (including pH, specific gravity, and bioactive marker quantification) into AYUSH regulatory protocols will enhance quality assurance and consusmer confidence in Ayurvedic formulations. Future research should focus on developing advanced stabilization strategies such as refrigerated concentrates, freeze-dried powders, or vacuum-sealed decoction sachets to retain therapeutic potency while improving convenience and shelf-life. Collectively, this study reaffirms that the scientific application of storage technology, analytical validation, and regulatory compliance can transform *Kwatha* preparations from perishable classical decoctions into reliable, evidence-backed formulations suitable for global Ayurvedic practice.

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