

EXPERIMENTAL STUDY

Standardization of *Khadradi Yoga Churna*, Mentioned in *Sahasra Yogam* with Physicochemical and Phytochemical Analysis

SMPPS Kumara¹, NDN Jayawardhane², HSV Jayasuriya^{3*}, PKD Chathurika⁴

¹Officer in-Charge, Ayurveda Hospital, Polwatta, Ambalangoda, Sri Lanka.

²Senior Lecturer, Department of Ayurveda Medicine and Indigenous Medicine, Faculty of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

³MD Scholar, Department of Ayurveda Roga Nidana Evim Vikriti Vigyana, National Institute of Ayurveda, Deemed to be University, Jaipur, Rajasthan, India.

⁴Demonstrator, Department of Ayurveda Medicine and Indigenous Medicine, Faculty of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

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ABSTRACT

Standardization of the herbal preparation is the process of standardizing the quality, consistency, and efficacy of products, aiming to evaluate their therapeutic properties. Analyzing the composition of herbal formulations is crucial for evaluating the quality, purity, safety, and effectiveness of drugs, as determined by the levels of their active ingredients. The current study aimed to establish a comprehensive pharmaceutical and analytical profile of the Ayurvedic poly-herbal *Khadradi Yoga churna*, as described in *Sahasra Yogam* which contain 05 herbal ingredients. Raw material identification done and preparation of medicine was done according to authentic text. Organoleptic evaluation, physicochemical analysis, phytochemical analysis, Chromatographic tests, Heavy metal test, Microbial test were conducted according to Ayurveda Pharmacopoeia of India (API) guidelines. Under quality and pharmacological significance, organoleptic evaluation showed traditional sensory attributes (color, odor, and taste). And under physicochemical key parameters; pH, moisture content, and total ash counts, extractive values tests were conducted and results align with API standards. glycosides, saponins, and phenolic compounds were identified as dominant compounds of the final product. TLC and HPTLC fingerprinting confirmed the presence of the main 06 ingredients, validating traditional composition. Absence of pathogens such as *E. coli*, Salmonella, comply with international standards and reflecting adherence to GMP. A critical safety concern was identified in heavy metal tests, regarding mercury contamination (Hg) 3.4 mg/kg) which is exceeding API acceptance limits ($\leq 1\text{mg/kg}$). Based on final results, while the *Khadradi yoga churna* demonstrates and fulfil excellent phytochemical and microbial quality parameters, Hg contamination overshadows its therapeutic potential. Further market analysis for ingredients, environmental contamination analyses or analysis of manufacturing procedures should be conducted to identify root cause for high mercury contamination.

1. INTRODUCTION

Standardization of the herbal preparation is the process of standardizing the quality, consistency, and efficacy of products, aiming to evaluate their therapeutic properties. The process of standardization includes various methodologies and parameters for measuring and isolating the active ingredient, contaminants, and quality attributes of herbal products. The World Health Organization (WHO) has also published Guidance on Quality Control Methods for Medicinal Plant Materials in 1992^[1] with the specific aim of providing general test methods for

proper botanical assessment and identification of medicinal plants commonly used in traditional and household remedies.

As a disease that causes social, psychological, and metabolic problems, obesity is one of the biggest public health concerns in the world. In addition to psychopathological illnesses such as depression and binge eating, it also raises the risk of cardiovascular diseases, diabetes, some types of cancer, high blood pressure, respiratory problems, disorders of the locomotor system, and dyslipidemia. Treating this is quite challenging because it takes a long time for the medication to take action. *Khadradi yoga* is a most suitable medication for that since it has very few ingredients and is very commonly used in Indian practice, and also those ingredients are commonly available in Sri Lanka. It is very essential to do studies

Corresponding Author:

HSV Jayasuriya,
National Institute of Ayurveda, Deemed to be University,
Jaipur, Rajasthan, India.
Email: shehanvidurangaja@gmail.com

on this kind of medicine to introduce simple ways to control globally burdened diseases.

Analyzing the composition of herbal formulations is crucial for evaluating the quality, purity, safety, and effectiveness of drugs, as determined by the levels of their active ingredients.^[2] The current study aimed to establish a comprehensive pharmaceutical and analytical profile of the Ayurvedic polyherbal *Khadiradi Yoga*, as described in *Sahasra Yogam*. This formulation consists of five medicinal herbs and is used to treat various ailments, providing a ready reference for quality assessment.

2. MATERIALS AND METHODS

2.1. Raw Materials Procurement and Authentication

Khadiradi yoga is a special *churna* (powder) mentioned under *Kashaya Prakarana* of *Sahasra Yogam*, an authentic Ayurvedic text that is most popular in Kerala, India [Table 1]. Raw materials were purchased in dried form from the local market, Pettah, Sri Lanka. Raw material identification and authentication have been done at the Laboratory of the Department of Ayurveda Pharmacology, Pharmaceutics, and Community Medicine, Faculty of Indigenous Medicine, University of Colombo.

2.2. Method of Preparation

The raw herbs (*Terminalia chebula*, *Terminalia bellirica*, *Phyllanthus emblica*) were cleaned individually and ground into a coarse powder using a pulverizer in the pharmacy unit of the Department of Ayurveda Pharmacology, Pharmaceutics, and Community Medicine at the Faculty of Indigenous Medicine, University of Colombo. The powder was then carefully sieved through an 85-mesh sieve to obtain a fine consistency. Finally, the powdered ingredients were stored in clean, round, airtight glass containers with silica gel packets to prevent moisture absorption. The *Kashaya* (decoction) was prepared by combining 04 parts (960 g) of *Khadirasara* and 04 parts (960 mL) of *Asanasara* with 12 parts (2880 mL) of water. The mixture was boiled over low heat until reduced to 480 mL. Subsequently, equal quantities (100 g each) of the ground herbal powders were blended and triturated (*Bhavana*) with the prepared decoction.^[3] The mixture was then dried and processed into a fine *churna* (powder). Finally, the powder was stored in an airtight glass container for preservation.

2.3. Analytical Parameters

2.3.1. Organoleptic evaluation

Organoleptic evaluation was performed to identify characteristics of the final product such as color, odor, taste, size, shape, and texture.

2.3.2. Physicochemical evaluation

The various standardization parameters were performed and studied according to globally accepted guidelines which are mentioned for herbal medicine such as Moisture content (loss of drying),^[4] Ash values (Total Ash, Acid insoluble ash, Water Soluble Ash),^[5] determination of extractive values^[6] (alcohol soluble extractive value [ASEV], water soluble extractive value [WSEV]), pH.^[7]

2.3.3. Phytochemical screening^[8]

The standard phytochemical identification tests performed such as Dragendorff's test, Mayer's test and Hager's test for identify Alkaloids, Molisch's test and Fehling's Test for Carbohydrates and Reducing Sugars, saponification test Glycosides, Ferric chloride test, Lead tetra acetic acid test for phenolic compounds and tannins, Alkaline reagent test, and Shinoda's test for flavonoids.

2.3.4. Chromatographical analysis

The thin layer chromatography (TLC) analysis was performed using a ternary solvent system of dichloromethane: cyclohexane:ethyl acetate (4:2:1 v/v/v) and a pre-coated silica gel 60G F254 aluminum plate for optimal compound separation. The TLC chamber was saturated for 10 min after adding the solvent system to a 5 mm depth. And both the test sample and reference standard dichloromethane extracts were carefully applied as 5 μ L spots 1 cm from the plate's bottom edge. The plate was developed vertically in the saturated chamber until the solvent front reached 8 cm from the origin. Then, for initial compound visualization, plate was air-dried and examined under UV light at 254 nm and 366 nm wavelengths. Standardized methodologies were followed to ensure reliable phytochemical profile comparison between the sample and reference materials.

High-performance TLC (HPTLC): Developed TLC plate was scanned with the HPTLC scanner using winCATS software.

2.3.5. Determination of Heavy Metals

Heavy metal analysis tests were conducted in compliance with the guidelines of Ayurvedic Pharmacopoeia of India (API). According to API standards, the acceptable limits for toxic elements (Arsenic [As] ≤ 3 μ g/g, Cadmium [Cd] ≤ 0.3 μ g/g, Mercury [Hg] ≤ 1 μ g/g, and Lead [Pb] ≤ 10 μ g/g).^[9,10]

Microbial contamination test was done for isolation of Enterobacteriaceae and certain other Gram-negative bacteria (*Escherichia coli*, *Salmonella* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*), Total Aerobic microbial count.^[11,12]

3. RESULTS

Organoleptic evaluation characteristics of *Khadiradi Yoga* were dark brownish, very fine powder appearance, bitter and aromatic mixed odor, Astringent (*Kashaya rasa*) and Bitter (*Tikta Rasa*) taste, and dry smooth texture, sieve size 80.

According to standard methods and levels, phytochemical analysis of *Khadiradi yoga churna* showed an acceptable pH level for oral medication. *Khadiradi Yoga* was slightly acidic pH (6.43 ± 0.07) when dissolved in water, which is acceptable for oral formulation and suitable for most herbal preparations. The moisture content was $8.22 \pm 0.04\%$. The total ash content was ($2.91 \pm 0.13\%$) and acid insoluble ash content was ($0.12 \pm 0.03\%$), Water Soluble Ash content was (1.71 ± 0.0578) (% w/w). Water soluble extractive value was (WSEV) ($26.15 \pm 0.09\%$) and alcohol soluble extractive value was (ASEV) ($22.81 \pm 0.04\%$) [Table 2, fig 1].

Screening for isolated phytochemicals of *Khadiradi yoga* revealed the presence of several bioactive compounds including glycosides (very high), tannins (very high), saponin (very high), carbohydrates and reducing sugars (high), volatile oils (low), each contributing uniquely to the medicinal properties of the herbal powder [Table 3].

HPTLC fingerprinting profile of *Khadiradi yoga churna*, at 360 nm wavelength bands, was visible at maximum R_f 0.04, 0.48, 0.50, 0.54, 0.63, 0.78 [Figure 2]. And corresponding colors (purple/yellow after derivatization) matched the standard's profile for *Bibhitaki* (*Terminalia bellirica*), *Haritaki* (*Terminalia chebula*), *Amalaki* (*Phyllanthus emblica*), *Khadira* resin (*Acacia catechu*), and *Asana* latex (*Pterocarpus marsupium*).

Under safety profile analysis, detected mercury content level of the sample was 3.4 mg/kg, Pb (lead) content level was 0.33 mg/kg and As

(arsenic) content level was 1.1 mg/kg were within acceptable limits, and Cd (cadmium) was not detected [Table 4]. Microbiological test results of *Khadiradi yoga choorna* showed favorable results, with the total aerobic microbial count 2.5×10^3 CFU/g, which is well below the WHO and API acceptance limits and absence of any kind of pathogens [Table 5].^[13]

4. DISCUSSION

With organoleptic evaluation, *Khadiradi yoga churna* aligned with smooth powder parameters according to traditional descriptions. pH value of an oral drug indicates the active components and it directly affects stability, solubility, and patient tolerance to the particular medicine. According to the WHO quality guidelines, the recommended pH range for oral herbal preparations is 5.0–7.0 (slightly acidic to neutral)^[14] except few formulations such as *Asava*, *Arishta*, and *Arka*. This slightly acidic nature of the medicine prevents degradation of pH-sensitive compounds in it such as alkaloids, glycosides. Moreover, it causes for minimizes gastrointestinal irritation since it is compatible with normal pH of human saliva (6.2–7.4). Moreover, it ensures compatibility with mucosal membranes apart from solubility and stability.

As global acceptance, if the moisture content level >10% promotes microbial (bacterial/fungal) growth, leading to spoilage. According to the results, *Khadiradi yoga* had low levels of moisture content and it indicates good storage stability with low risk of microbial growth. Moisture content test is an important quality control parameter for herbal powders, and it measures the moisture content and volatile components of particular drug. It plays major role in maintaining stability and shelf life of a product, specially controlling microbial growth. The consistent moisture content results of the powder across all samples indicates uniformity in processing and storage conditions.

Ash values of a medicinal powder indicate minimal chances for contamination with inorganic matter such as siliceous impurities or soil and they confirm the purity of the powder. Ash values quality control parameters of herbal medicines provide insights into test drug's purity, mineral content, and potential contamination. Normally total ash value represents the total inorganic residue (minerals, silica, etc.) after incineration. High ash contents indicate adulteration presence of the medicine. According to sample results, it shows significant low levels of total ash value compared to total ash limitations for herbal powder of API guidelines (<6%). Acid-insoluble ash content measures contaminants of silica, sand, or other indigestible. According to the results of the study, it shows low levels of acid insoluble ash content comparatively to API (<1%) guidelines for herbal powder.

The prominent phytochemicals such as glycosides, tannins, and saponins correlate with *Khadiradi Yoga's* traditional uses, such as anti-inflammatory and antimicrobial effects. HPTLC validated the presence of all claimed ingredients, ensuring formulation integrity. The dominance of polar compounds correlates with high water-soluble extractive values, ensuring good bioavailability in aqueous preparations.

Extractive values of the *Khadiradi Yoga* suggest a significant presence of both polar and non-polar phyto-constituents such as sugars, glycosides, and tannins. This directs to making the extract suitable for further pharmacological profiling. High consistency of extractive values suggests standardized processing and raw material quality. According to API guidelines, Ayurvedic *churnas* (powders) require

$\geq 15\%$ ASEV. Test results show higher values, confirming richness in alcohol-soluble actives. It indicates the bioavailability of fat-soluble compounds. According to API guidelines, minimally, it requires $\geq 20\%$ WSEV for herbal powders. Test results show strong water-soluble polar bioactive compounds (sugars, glycosides, tannins, and amino acids).

In heavy metal analysis, the detected mercury level of 3.4 mg/kg significantly exceeds API permissible limits for mercury present in herbal medicine (≤ 1 mg/kg). It will pose serious health risks, including potential neurotoxicity and severe renal damage. This test result suggests possible environmental contamination during cultivation, storage, adulteration, or processing. Pb (lead) 0.33 mg/kg and as (arsenic) 1.1 mg/kg were within acceptable limits, and Cd (cadmium) was not detected. The mercury contamination alone renders the product unsafe for consumption according to international quality standards including WHO and API guidelines. These findings emphasize the need for adherence to quality control measures in herbal product manufacturing, especially supply chains, particularly in regions where soil and water contamination may be prevalent. The absence of pathogenic microorganisms including *Staphylococcus aureus*, *E. coli*, and *Salmonella* spp. indicates proper hygienic protocol adherence from the beginning.

HPTLC fingerprinting profile of *Khadiradi yoga churna*, when developed using a single mobile phase system, demonstrated distinct band formations for each individual component drug. When compared to the complete compound formulation's chromatographic profile, it clearly confirmed the presence of all constituent single drugs in the final preparation.

5. CONCLUSION

Analysis of *Khadiradi Yoga Churna* encompasses international standardization parameters, including physicochemical, phytochemical, heavy metal, and microbial assessments. Collectively, these tests provide a holistic understanding of drug quality, safety, and therapeutic potential in Ayurvedic practice. Under quality and pharmacological significance, organoleptic evaluation showed traditional sensory attributes (color, odor, and taste). And under physicochemical key parameters (pH, moisture content, and total ash counts [total ash, acid-insoluble ash, and water-soluble ash]) were checked and they align with API standards. That ensures formulation stability. High water-soluble extractive values and alcohol-soluble extractive values indicate rich bioavailability of active constituents. With phytochemical-rich outcomes such as dominant identification glycosides, saponins, and phenolic compounds, it shows the ability for vast therapeutic applications in clinical practice. TLC and HPTLC fingerprinting confirmed the presence of the main 06 ingredients, validating traditional composition. Absence of pathogens such as *E. coli*, *Salmonella*, comply with international standards and reflecting adherence to GMP. A critical safety concern was identified in heavy metal tests, regarding mercury contamination (Hg) 3.4 mg/kg which is exceeding API acceptance limits (≤ 1 mg/kg). High mercury levels can lead to severe risk of neurotoxicity (CNS damage) as well as nephrotoxicity (kidney dysfunction) and potential accumulation with prolonged use. While the *Khadiradi yoga churna* demonstrates and fulfills excellent phytochemical and microbial quality parameters, Hg contamination overshadows its therapeutic potential. Because of that, the root cause should be identified with market analysis for ingredients, environmental contamination analyses, or analysis of manufacturing procedures.

6. ACKNOWLEDGMENTS

Nil.

7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

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9. ETHICAL APPROVALS

This study does not require ethical clearance as it is a case study.

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAILABILITY

This is an original manuscript, and all data are available for only review purposes from the principal investigators.

12. PUBLISHERS NOTE

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Table 1: Ingredients, composition, and part of use of *Khadiradi yoga*

Sanskrit name	Scientific name	Used part	Composition
<i>Khadirasara</i>	<i>Acacia catechu</i>	Exudation of	300 g
<i>Aanasara</i>	<i>Pterocarpus marsupium</i>	latex	300 mL
<i>Haritaki</i>	<i>Terminalia chebula</i>	Fruit cover	100 g
<i>Bibhitaki</i>	<i>Terminalia bellirica</i>	Fruit	100 g
<i>Amalaki</i>	<i>Phyllanthus emblica</i>	Fruit	100 g

Table 2: Physicochemical evaluation of three samples of *Khadiradi yoga churna* (values are percentage mean determination $\pm$ standard error of the mean)

Test	Sample 1	Sample 2	Sample 3	Results
Total ash (% w/w)	3.12	2.67	2.95	2.91 $\pm$ 0.1312
Acid insoluble ash (% w/w)	0.10	0.08	0.18	0.12 $\pm$ 0.0306
Water soluble ash (% w/w)	1.70	1.61	1.81	1.71 $\pm$ 0.0578
Alcohol soluble extractive (% w/w)	22.88	22.76	22.80	22.81 $\pm$ 0.0353
Water soluble extractive (% w/w)	26.20	25.98	26.26	26.15 $\pm$ 0.0851
Loss on drying (% w/w)	8.20	8.30	8.16	8.22 $\pm$ 0.0416
pH (1% aqueous solution)	6.5	6.3	6.5	6.43 $\pm$ 0.0667

w/w: Weight per weight

Table 3: Phytochemical analysis of three samples of *Khadiradi yoga churna*

Phytochemical test	Sample 1	Sample 2	Sample 3	Inference
Volatile oils	+	+	+	Present
Alkaloids (Dragendorff/Mayer/Hager)	-	-	-	Absent
Carbohydrates (Molisch's)	++	++	++	Moderate
Reducing Sugars (Fehling's)	+++	+++	+++	Strong
Glycosides	+++	+++	+++	Strong
Phenolic compounds and tannins	+++	+++	+++	Strong
Flavonoids (Alkaline/Shinoda's)	-	-	-	Absent
Saponins	+++	+++	+++	Strong

Table 4: Heavy metal analysis of *Khadiradi yoga churna*

Metal	Result	Limit of Determination	Interpretation
Lead (Pb)	0.33 mg/kg	-	Low level; below WHO/API limits (typically 10 mg/kg for herbs).
Cadmium (Cd)	Not detected	0.05 mg/kg	Undetectable, indicating no cadmium contamination.
Arsenic (As)	1.1 mg/kg	-	Moderate; verify against regional limits (WHO/API allows 3 mg/kg).
Mercury (Hg)	3.4 mg/kg	-	High risk; exceeds typical limits (WHO/API limit: 1 mg/kg). Requires further action.

WHO: World Health Organization

Table 5: Microbial analysis of *Khadiradi yoga churna*

Parameter	Result	Interpretation
Total aerobic microbial count (TAMC)	2.5×1032.5×103 CFU/g	Within acceptable limits for herbal products (typically<104104 CFU/g).
Pathogens	Absent (<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Salmonella</i> , etc.)	No harmful bacteria detected, meeting safety standards.

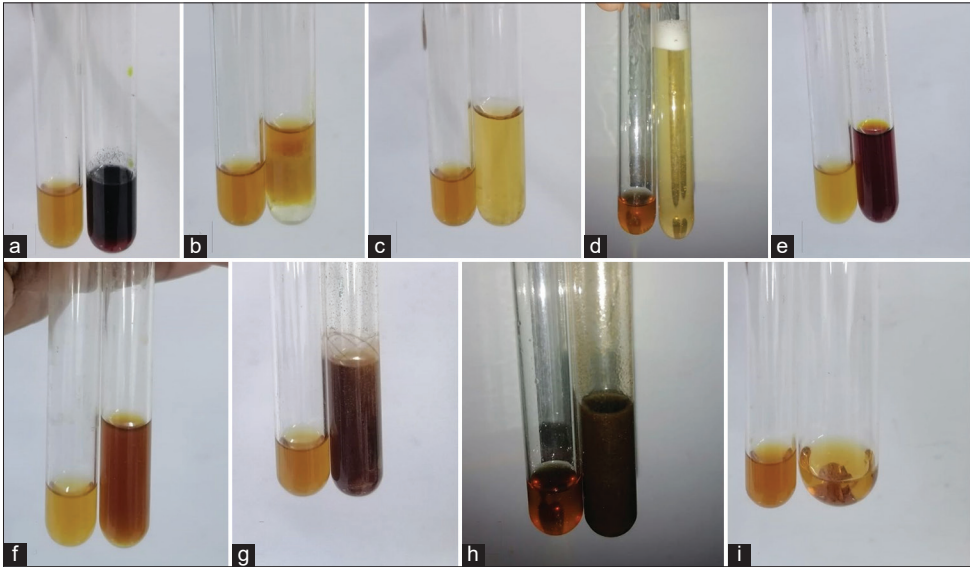


Figure 1: Phytochemical analysis for isolation of (a) Tannin, (b) Tannin, (c) Alkaloids (Negative), (d) Saponin, (e) Alkaloids (negative), (f) Flavonoids (negative), (g) Reducing sugar, (h) Carbohydrates, (i) Flavonoids (Negative)

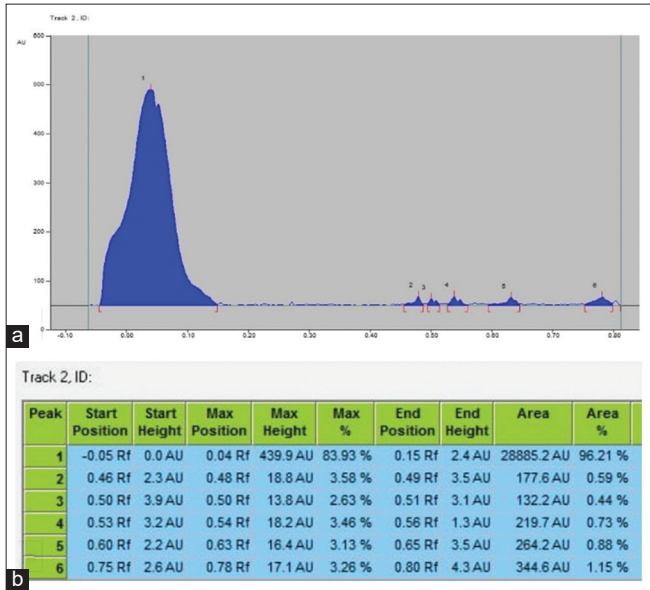


Figure 2: High-performance thin-layer chromatography (HPTLC) Fingerprint, (a) Observed peak for Rf values at 366 nm, (b) HPTLC fingerprint of the dichloromethane extract of the final product