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Pharmacognostic standardization and HPTLC fingerprinting analysis of *Sahacharadi Kashayam*: a classical ayurvedic polyherbal formulation

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ABSTRACT

Multiherbal compositions are widely practiced in ayurvedic medical system, and kaṣāyam (decoction) is one such dose form. Sahacharadi Kashayam is a traditional ayurvedic polyherbal medicine recommended by ayurvedic doctors for chronic and acute back pains, lower limb disorders, and conditions that mainly affect the nutrient level in the blood and the bone. The aim of this study was to develop pharmacognostic parameters for the standardization and quality evaluation of the *Sahacharadi Kashayam.* In-house polyherbal formulation was prepared as per the previously prescribed method, and physicochemical parameters were evaluated as per standard protocol and showed loss on drying at 99.31%, total ash content at 4.911 ± 0.120%, water-soluble ash at 3.022, and acid-insoluble ash at 0.489. Total phenolic content was estimated with FC reagent and expressed as gallic acid equivalent (9.95 + 0.004 mg/g). High-performance thin-layer chromatography (HPTLC) fingerprint profiling was obtained, and the quantity of total tannins was estimated as tannic acid equivalent using HPLC (0.47%). The results obtained from the study could be utilized as a reference for the quality control and quality assurance of *Sahacharadi Kashayam*.

1. INTRODUCTION

Multiherbal compositions such as medicated oils, ghee, decoctions, and the likes are widely practiced by ayurvedic doctors. In ayurveda, one such dose form that is widely used is *kaṣāyam* (decoction) [1]. *Kashayam* (also known as *kwath*) is a concentrated decoction prepared from herbal ingredients consisting of water-soluble active principles. *Sahacharadi Kashayam* is a classical ayurvedic polyherbal formulation prescribed by ayurvedic physicians for chronic and acute back pains, lower limb disorders, and conditions that mainly affect the nutrient level in the blood and the bone. The formulation is prepared by combining the three plants: *Barleria prionitis, Cedrus deodara,* and *Zingiber officinale* [2,3].

Compared to their single herbal equivalents, polyherbal formulations present a greater difficulty in terms of establishing their quality, efficacy, and safety due to their complexity. As a result, it is critical to standardize herbal medications using a variety of criteria and advanced methods to guarantee their efficacy, safety, and quality [4,5]. The recommendations for standardizing herbal medicines have been provided by a number of regulatory authorities, including the Department of AYUSH, Government of India, the European Agency for the Evaluation of Medicinal Products (EMEA), the World Health Organization (WHO), and the United States Pharmacopeia (USP) [6,7].

In the present work, we have developed the quality control parameters such as physicochemical parameters, HPTLC fingerprints, and HPLC quantification of marker compounds for authentication and routine quality control of *Sahacharadi Kashayam.*

2. MATERIALS AND METHODS

2.1. Materials

HPLC and analytical-grade chemicals were purchased from the commercial supplier and used in the study.

2.2. Procurement of Plant Material

The dried plant materials were purchased from verified commercial suppliers and authenticated at the Department of Pharmacognosy, BVM College of Pharmacy, Gwalior, India, and a voucher specimen was submitted to the institute. The samples were visually inspected and validated for their morphological traits, then powdered using a

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mechanical blender at very low speed, and the powdered samples were stored in airtight containers and kept in a dry place away from sunlight.

2.3. Preparation of *Sahacharadi Kashayam*

The dried powder of plants *Barleria prionitis, Cedrus deodara,* and *Zingiber officinale* were mixed in a previously described proportion [Table 1] to obtain the dried powder of *Sahacharadi Kashayam*, and the aqueous decoction (kashayam/kwath) was prepared as described by Nisteshwar and Vidyanath [2]. The prepared kwath solution was concentrated under vacuum to obtain a semisolid mass, which was further dried at 40°C to obtain a powdered formulation and stored in airtight containers in a dry place away from sunlight, until further use.

2.4. Physicochemical Evaluation, Total Phenolic Contents, and DPPH Radical Scavenging Assay

Physicochemical parameters such as total ash content, water-soluble ash, acid-insoluble ash, loss on drying of the extract, and pH were carried out on the dried extract, according to the standard protocols [8,9]. Total phenolic content was spectrophotometrically estimated using Folin–Ciocalteu reagent assays at 750 nm as per the method described by Anunthawan and Rimlumduan [10]. Gallic acid was used as a standard, and the total phenolic contents were expressed as gallic acid equivalents. DPPH radical scavenging assay was performed as per the method described by Molole et al. [11]; in brief, 0.4 mL of test sample (SK) and gallic acid (50–400 µg/mL) were mixed with 2.6 mL of a methanolic solution of DPPH (0.1 mM). An equal amount of methanol (0.4 mL) and DPPH solution (2.6 mL) was used as a blank. The samples were all produced in triplicate, vortexed for 1 min, and then incubated at 37°C in the dark for 30 min. The decrease in absorbance of each sample was measured against methanol as a blank on a UV–visible spectrophotometer at 517 nm. All the experiments were performed in triplicate, and the results were expressed as mean ± SD.

2.5. HPTLC Method

The dried aqueous extract of *Sahacharadi kashayam* was dissolved in water and subjected to HPTLC analysis. A total of 10 µL of the extract was spotted on HPTLC silica gel 60F 254 (Merck) plate as bands of 6-mm length. The plates were developed using toluene: ethyl acetate: methanol: formic acid (16:14:1:4) in the CAMAG twin-trough glass chamber previously saturated with the solvent for 30 min. After development, the plates were oven dried at 60°C and scanned at 254 nm and 366 nm.

2.6. Estimation of Tannic Acid Content Using HPLC

A total of 5 g of dried extract powder of SK was dissolved in HPLC-grade methanol to make a 10 mg mL^{-1} solution, and the samples were sonicated for 15 min and filtered through a 0.45-µm Millipore nylon filter. The HPLC-grade tannic acid (96% purity) purchased from HiMedia Laboratories Pvt Ltd, Mumbai, India, was used as the standard in a concentration of 10 µg mL^{-1}. RP-HPLC (Shimadzu Scientific Instrument, Kyoto, Japan) equipped with a stainless steel column 25 cm × 4.6 mm packed with octadecylsilane-bonded porous silica (5 µm) and SPD40 UV–Vis detector was used for analysis. The column temperature was kept at 40°C and the injection volume was 20 µL. The mobile phase consisted of 0.1% formic acid in water in pump A and methanol in pump B, both at a flow rate of 0.7 mL min^{-1}, and eluted with solvents A and B at a ratio of 95:5, respectively, for 10 min, adding 100% methanol for the next 5 min, and absorbance monitoring was done at 272 nm.

Table 1: Composition of *Sahacharadi Kashayam.*

S. No.	Vernacular Name	Botanical Name	Quantity (g)
1	Sahachara	*Barleria prionitis*	9.260
2	Suradaru (Devadaru)	*Cedrus deodara*	6.173
3	Shunthi	*Zingiber officinale*	3.086

3. RESULTS AND DISCUSSION

3.1. Physicochemical Evaluation and Total Phenolic Contents

Physicochemical parameters such as total ash content, water-soluble ash, acid-insoluble ash, loss on drying, and pH including total phenolic content were evaluated for SK and the results have been summarized in Table 2.

3.2. DPPH Radical Scavenging Assay

SK indicated a good antioxidant activity in terms of percentage inhibition (68.30%) of DPPH radical [Figure 1], which is comparable to the study of Kumar et al. [3].

3.3. HPTLC Figure Printing

The HPTLC fingerprinting profile was developed in toluene: ethyl acetate: methanol: formic acid (16:14:1:4) and the HPTLC chromatogram showed a total of eight bands, and the corresponding peaks were recorded at respective Rf values as depicted in Figure 2.

Table 2: Physicochemical analysis of *Sahacharadi Kashayam*.

S. No.	Physicochemical Parameters	Results*
1	Total ash content	4.911 ± 0.20
2	Water-soluble ash	3.022 ± 0.120
3	Acid-insoluble ash	0.489 ± 0.032
4	LOD at 105°C	6.31 ± 0.36
5	pH in aqueous solution	6.1 ± 0.3
6	Total phenolic content	9.95 + 0.004 mg/g GAE

*Values were expressed as a percentage of dried extract. TPC expressed as mg/g gallic acid equivalent.

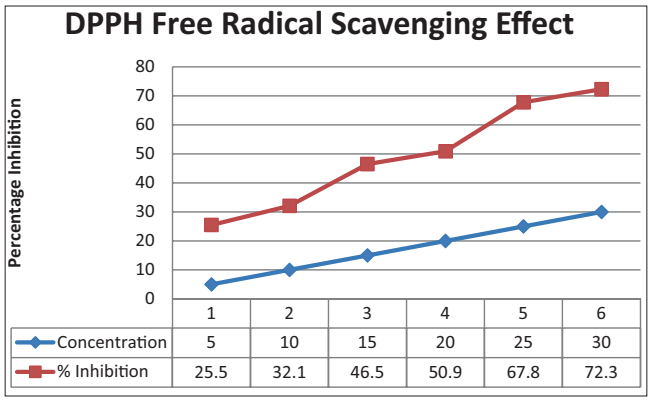
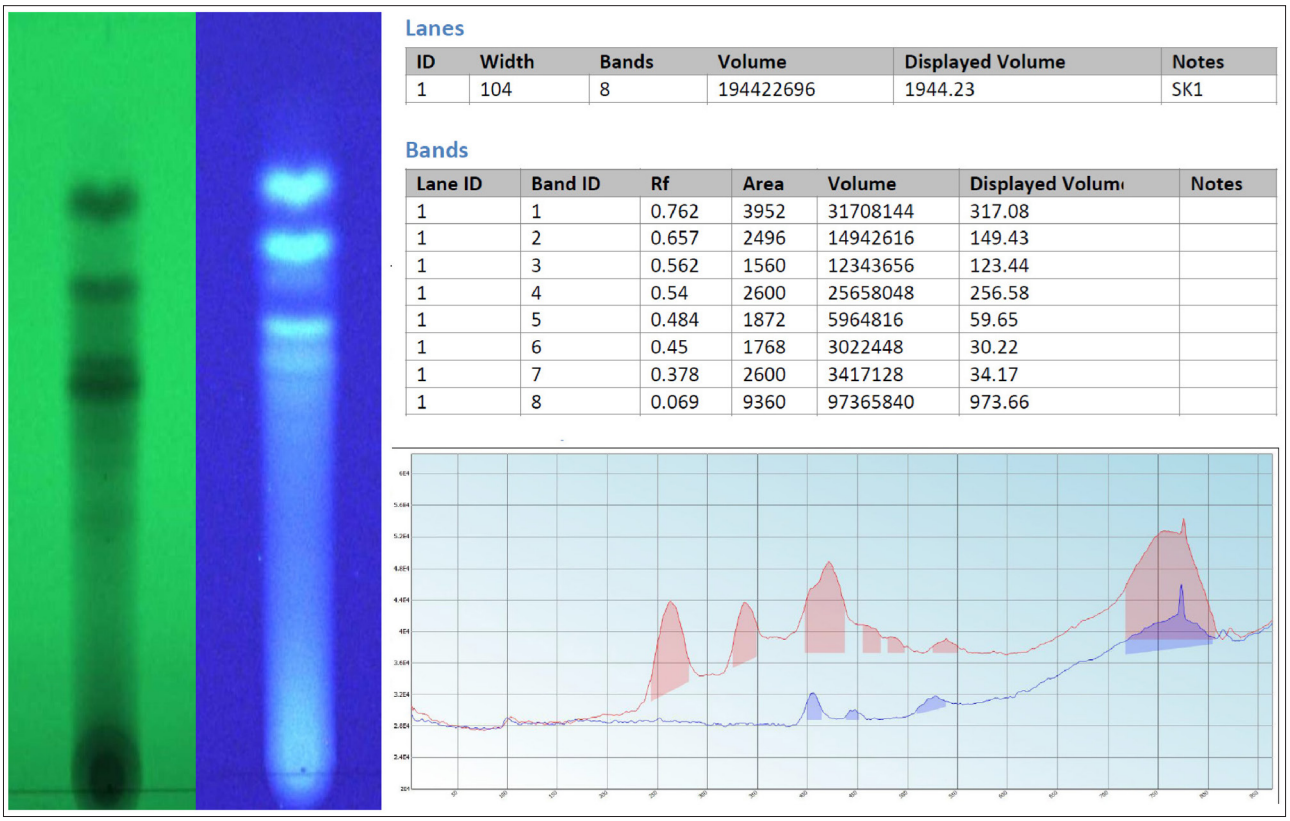


Figure 1: DPPH Free radical scavenging effect of *Sahacharadi Kashayam*.



Lanes

ID	Width	Bands	Volume	Displayed Volume	Notes
1	104	8	194422696	1944.23	SK1

Bands

Lane ID	Band ID	Rf	Area	Volume	Displayed Volum	Notes
1	1	0.762	3952	31708144	317.08	
1	2	0.657	2496	14942616	149.43	
1	3	0.562	1560	12343656	123.44	
1	4	0.54	2600	25658048	256.58	
1	5	0.484	1872	5964816	59.65	
1	6	0.45	1768	3022448	30.22	
1	7	0.378	2600	3417128	34.17	
1	8	0.069	9360	97365840	973.66	

Figure 2: HPTLC fingerprint profile of tannins in leaf extracts of *Sahacharadi Kashayam.*

Table 3: The linear regression values for the method.

Compound	Regression Equation (Y)	Correlation Coefficient (R^2)	λ	Rt	Detected Quantity	%RSD
Tannic acid	0.447	0.998	272	6.118	0.47 mg/g	1.08

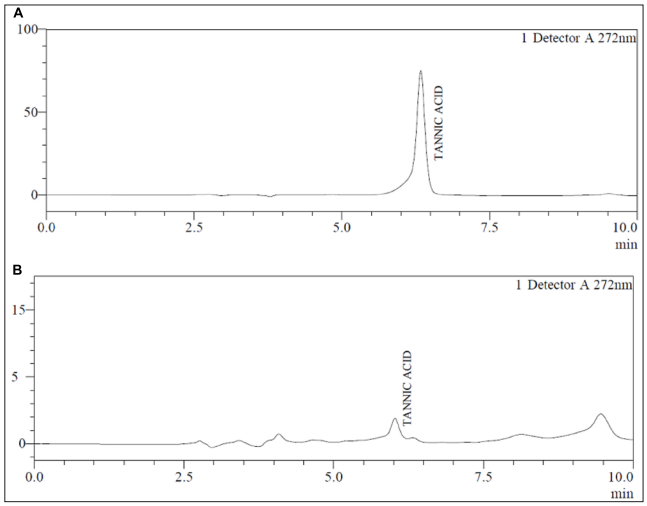


Figure 3: HPLC chromatogram of **(A)** standard tannic acid and **(B)** aqueous extract of *Sahacharadi Kashayam.*

3.4. Estimation of Tannic Acid Content Using HPLC

HPLC analysis of the aqueous extract of SK was carried out along with standard tannic acid. The SK showed a characteristic peak corresponding to tannic acid at 6.118. The calibration curve (peak area vs concentration) of serially diluted tannic acid was obtained from the analysis at 272nm and has shown good linearity in the tested range. The linear regression values for the method are shown in Table 3, and chromatograms of the standard and sample are shown in Figures 3A and 3B, respectively.

4. CONCLUSION

The present work is an attempt to develop the qualitative and quantitative standardization parameters for *Sahacharyadi Kashayam*. The results of this research can be used to monitor day-to-day quality control process during the manufacturing of these formulations to avoid the product-to-product and batch-to-batch variations and to maintain the quality of finished formulations. The fingerprints developed using HPTLC and HPLC can be used for identification and quantification of the marker and will also be helpful in determining adulteration for SK preparations.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval

of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

12. PUBLISHER'S NOTE

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