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Analysis of quality parameters and preservative concentrations in Sahacharadi Kwatha: A comparative study of three commercial brands

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ABSTRACT

Background: Sahacharadi Kwatha is traditionally employed in Ayurvedic therapy for "vata" related conditions such as back pain, herniated disc, palsy, sciatica, and paralysis. Classical Ayurvedic texts recommend the use of freshly prepared Kwatha for optimal patient benefits. However, in response to the commercialization of Ayurveda and the demand for convenient over-the-counter (OTC) formulations, various commercial preservatives have been incorporated by Ayurvedic manufacturers to facilitate OTC preparation and prolong shelf life.

Objectives: This study aims to comprehensively analyse and compare the quality parameters and preservative content in three prominent brands of *Sahacharadi Kwatha* available in the Indian market.

Materials and methods: Organoleptic and physicochemical properties, phytochemical content, and microbial load of the samples were analyzed following standardized procedures. Sodium benzoate levels in the samples were determined using both titrimetric and High-Performance Liquid Chromatography (HPLC) methods. High-Performance Thin-Layer Chromatography (HPTLC) profiles were compared to discern differences among the samples.

Results: The study revealed significant variations in organoleptic and physicochemical properties, HPTLC profiles, and microbial load among the tested samples. Sodium benzoate levels in all samples exceeded the FDA and API-approved limit. Additionally, substantial variations were noted in the phytochemical content of the samples.

Conclusion: This investigation underscores noteworthy disparities in quality parameters and preservative content within the tested market variants of *Sahacharadi Kwatha*. The findings emphasize the existence of unregulated standards in the preparation of Ayurvedic medicines available in the market, highlighting the imperative for standardization and validation of Ayurvedic formulations. Such measures are essential for enhancing consumer acceptability and fostering the overall development and growth of the Ayurveda industry.

1. Introduction

The traditional Indian medical system known as Ayurveda is a very popular among the Indian population [1]. Ayurveda places a significant emphasis on polyherbal formulations, comprising medicinal preparations derived from diverse plant components, including roots, bark, leaves, flowers, seeds, and stems. Various parts of the herbs are completely utilized for their pharmacological effects and are processed into a variety of herbal preparations including *Hima* (Cold infusion), *Kwatha* (Decoction), *Phanta* (Hot infusion), *Arka* (Liquid Extract), *Churna* (Powders),Taila(Medicinal oil), *Guggul* (Resins and balsams)etc [2]. Polyherbal preparations are effective in small doses, safe even at higher doses, and have fewer side effects. When combined in the correct proportions, authentic herbal drugs interact synergistically, enhancing the therapeutic potential of polyherbal formulations [3].

Kwatha, an aqueous decoction derived from medicinal plants, represents a distinct category within methods of preparation of Ayurvedic formulations elucidated in Classical Ayurvedic texts, falling under the classification of *Panchavidha Kashaya Kalpana* [4]. *Kwatha* is prepared by boiling coarsely powdered medicinal drug in sixteen times of water until residual portion of liquid is reduced to one eighth of entire matter and is filtered [5].

Sahacharadi Kwatha, a widely recognized Ayurvedic decoction, comprises the herbal components *Sahachara* (*Barleria prionitis*), *Suradaru* (Devadaru) (*Cedrus deodara*), and *Sunthi* (*Zingiber officinale*) [6,7]. This decoction manifests notable anti-inflammatory and analgesic properties,

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with each botanical constituent playing a pivotal role in determining its distinct therapeutic efficacy. *Barleria prionitis*, for instance, demonstrates *Kapha* and *Vata*-reducing attributes coupled with antioxidant, anti-inflammatory, anti-arthritic, and cytoprotective activities [8–11]. *Cedrus deodara* exhibits immune-modulating, anti-inflammatory, anti-arthritic, analgesic, and antioxidant properties, while *Zingiber officinale* is renowned for its analgesic, anti-inflammatory, and antioxidant attributes [12–14].

The paradigm of polyherbal formulations is firmly ingrained in Ayurveda and other traditional medical systems, wherein the incorporation of multiple herbs in a singular preparation is acknowledged to confer advantages beyond those attainable with single herbal formulations. This approach is predicated on the principle of synergism, denoting a favourable interaction between distinct herbs. Practitioners contend that polyherbal formulations mitigate the risk of adverse effects associated with elevated doses of individual herbs and enhance the palatability of the preparations for patient consumption [15,16].

The escalating demand for Ayurvedic formulations signifies a burgeoning inclination towards natural and holistic approaches to health and wellness. This surge is propelled by various factors, including an increasing awareness of the limitations and potential side effects associated with conventional pharmaceuticals, a global shift towards preventive healthcare strategies, a personalized healthcare paradigm, and the mounting endorsement of Ayurveda by regulatory authorities and healthcare professionals [17–19].

Nevertheless, the proliferation of Ayurvedic formulations by numerous pharmaceutical entities has resulted in noticeable disparities among identical formulations, instigating apprehensions regarding the consistency of quality standards. To ascertain the safety, efficacy, and uniformity of these formulations, standardization becomes imperative. This becomes particularly salient when identical formulations are produced by multiple manufacturers. Adhering to international quality standards is pivotal for the acknowledgment and assimilation of Ayurvedic formulations into conventional healthcare practices [18,20].

Several studies underscore the exigency for quality control and standardization of Ayurvedic formulations. Notably, the establishment of quality control parameters for Ayurvedic formulations such as *Drakshārishta* and *Triphaladi* granules has been underscored. These endeavors are directed towards scrutinizing the quality, efficacy, and safety of Ayurvedic formulations, which manifest in various dosage forms, including solid, liquid, and semi-solid formulations. The formulation of standards for the quality and purity of raw materials, coupled with stringent quality control procedures throughout the manufacturing process, assumes paramount importance for the development of superior-quality finished Ayurvedic products. However, to guarantee the safety, efficacy, and uniformity of these formulations, adherence to international quality standards and rigorous quality control measures is indispensable. Initiatives to standardize Ayurvedic formulations are pivotal for their assimilation into mainstream healthcare practices and for fostering consumer confidence [17–20].

In view of rising demand for Ayurveda medicines and the challenges facing by the Ayurveda pharmaceutical industries, the current study aims to compare the quality parameters and preservative load of commercially manufactured *Sahacharadi Kwatha* from three prominent brands available in Kerala.

2. Materials and Methods

2.1. Sample collection

The *Sahacharadi Kwatha* specimens were obtained from duly authorized vendors located in the vicinity of Irinjalakuda, Thrissur district, Kerala, with a primary focus on upholding authenticity and dependability. Thorough scrutiny of the sample containers was conducted to ensure proper sealing and adherence to the stipulated expiration dates. To maintain confidentiality, the samples were designated as S1, S2, and S3. Additionally, a comprehensive assessment of the sample bottles encompassed verification of details pertaining to the herbal constituents, proportions of each herbal ingredient, references to classical Ayurvedic literature or authoritative texts utilized in the formulation of *Sahacharadi Kwatha*, and any indications of the presence of supplementary preservatives.

2.2. Organoleptic characterization

The methodology outlined by Siddiqui et al. (1995) was used to conduct the assessment of organoleptic attributes, including color, odour, taste, and consistency of the Kwatha using the sensory organs of our body. A panel of 10 members was selected randomly, and the samples were given to them for sensory analysis. Based on their feedback, the characteristics were reported. This methodology is a standard method for evaluating the physical, phytochemical, and chromatographic parameters of herbal formulations [21,22].

2.3. Physicochemical analysis

The quality standards were primarily evaluated by checking different physicochemical parameters including pH and TDS using a digital pH meter (Infra Instruments Pvt Ltd, Chennai; Model no: IR 50/A) and digital TDS meter (Esico International, Haryana; Model No: 651), respectively and the readings were recorded accordingly [17].

2.4. Microbial load analysis (total plate count)

The plate count method was used to determine the total aerobic microbial count, encompassing both bacteria and fungi [23,24].

2.5. Phytochemical analysis

2.5.1. Qualitative phytochemical analysis

The presence or absence of phytochemical components were assed via qualitative phytochemical screening assays which consists of a series of standard methods including Dragendroff's reagent test for alkaloids [25], Shinoda test for flavonoids [25], Lead acetate test for tannins [25], Picric acid test for glycosides [26], Foam test for saponins [25,27], Salkowski reaction test for terpenoids [27], Folin ciocalteu reagent test Phenol [28], Molisch's test for carbohydrates [29].

2.5.2. Quantitative phytochemical analysis

2.5.2.1. Estimation of total alkaloids. 20 ml of a 10% sodium hydroxide (NaOH) solution was combined with 5g of a Kwatha sample within a separating funnel. The resulting mixture underwent multiple additions of 20 ml of chloroform, accompanied by thorough shaking to facilitate the separation of alkaloids. The chloroform layer, containing the separated alkaloids, was subjected to sequential washing steps with water to eliminate impurities, followed by an acid wash utilizing 20 ml of 1 N hydrochloric acid (HCl). Subsequently, the final collected solvent layer underwent an addition of 15 ml of ammonia with continuous mixing. The chloroform layer, now enriched with alkaloids, was carefully collected, subjected to evaporation to dryness, and the percentage weight was determined using a prescribed formula [30,31].

$$\%\ \text{Alkaloid} = \frac{\text{Weight of residue}}{\text{Weight of sample}} * 100 \quad (1)$$

2.5.2.2. Estimation of flavonoid. 25 ml of methanol was mixed with 59.1 mg of the sample, and the resulting mixture was subjected to sonication for a duration of 20 min. Subsequently, 2 ml of the aforementioned mixture was blended with 1.5 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of sodium acetate, and 2.8 ml of distilled water. The resultant solution was incubated at room temperature for 30

min, and the absorbance was measured at a wavelength of 415 nm [32].

$$\%\ \text{Flavanoid} = \frac{\text{Observed concentration*Purity of standard}}{\text{Sample concentration}} \quad (2)$$

2.5.2.3. Estimation of phenol. 29.9 mg of the sample was mixed with 25 ml of distilled water and subjected to sonication for a duration of 20 min. Subsequently, 3 ml of the resulting mixture was combined with 4 mL of sodium carbonate solution and 1 ml of phenol reagent, and the volume was adjusted to 25 ml. The resultant solution underwent a further sonication period of 10 min and was then left in the dark for 30 min. The absorbance of the solution was measured at a wavelength of 760 nm [32].

2.5.2.4. Estimation of Saponin. 20g sample was initially mixed with 100 ml of 20% ethanol and subjected to heating over a water bath at 100 °C for a duration of 4 h with continuous stirring. The resulting residue, obtained through the filtration of the solution, underwent re-extraction using 200 ml of 20% ethanol. The combined extracts were subsequently reduced to a volume of 40 ml by heating in a water bath. Following this concentration step, 20 ml of diethyl ether was introduced into the concentrated extract, leading to the separation and recovery of the aqueous layer. To further process the extract, 60 ml of n-butanol was added, followed by two washes with 10 ml of 5% NaCl. The remaining solution underwent additional heating in a water bath and subsequent drying in an oven at 105 °C until a constant weight was achieved [33].

$$\%\ \text{Saponins} = \frac{\text{Residue Weight}}{\text{Weight of sample}} * 100 \quad (3)$$

2.5.2.5. Estimation of tannin. 1g sample was homogenously blended with 100 ml of distilled water. Subsequently, 25 ml of indigosulphonic acid, along with 75 ml of distilled water, was introduced into 10 ml of the prepared mixture. The resulting solution was titrated against a 0.1 N potassium permanganate solution, with a blank solution containing 5 ml of distilled water and 25 ml of indigosulphonic acid for reference. The titration values obtained from the sample and the blank were utilized to calculate the difference in milliliters between the two titrations [34].

$$\text{Amount of tannin} = \frac{(\text{Titre volume of sample} - \text{Blank titre volume}) * 004157 * 100}{\text{Weight of sample}} \quad (4)$$

2.5.2.6. Estimation of carbohydrates. The quantitative determination of total soluble carbohydrates was conducted using Anthrone's method, with D-glucose serving as the standard. The sample underwent hydrolysis in the presence of dilute hydrochloric acid (HCl), breaking down complex carbohydrates into simple sugars. In the acidic environment, glucose underwent dehydration, yielding hydroxyl methyl furfural. The ensuing compound reacted with anthrone to produce a green-colored product, exhibiting an absorption maximum at 630 nm [35].

$$\%\ \text{Carbohydrate} = \frac{\text{Observed concentration*Purity of standard}}{\text{Sample concentration}} \quad (5)$$

2.6. HPTLC profiling

One gram of the sample was solubilized in 5 mL of methanol, subjected to filtration, and subsequently analyzed using thin-layer chromatography (TLC). An aliquot of 2 µL from the obtained extract was loaded onto a pre-coated high-performance thin-layer chromatography (HPTLC) plate (5 × 10 cm, thickness - 0.2 mm) employing a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland). The stationary phase of the HPTLC plate comprised silica gel with aluminum sheet support (60 F254 TLC plates, E. Merck). The development of the plate was carried out to a distance of 8 cm using a mobile phase consisting of Toluene: Ethyl acetate: Formic acid in a ratio of 7:3:0.5. Following development, the plate was allowed to air-dry and subsequently examined under ultraviolet light at wavelengths of 254 nm and 366 nm. The resultant Rf values and colors of the resolved bands were documented and compared against the standard markers beta sitasterol, vasicine and gallic acid. The spotting device employed was a CAMAG Linomat V Automatic Sample Spotter, equipped with a 100 µL capacity Hamilton syringe and developed using the CAMAG glass twin trough chamber (5 × 10 cm). The densitometer employed for analysis was a CAMAG TLC scanner 3, linked to WINCATS software for data acquisition and processing.

2.7. Preservative load analysis

2.7.1. Titration

In a 250 ml volumetric flask, 8g of laboratory-grade NaCl (Brand: NICE) was dissolved in 50 ml of Kwatha sample. The solution was diluted with 150 ml saturated NaCl solution and made alkaline with 10% NaOH (Brand: NICE). After reaching a total volume of 250 ml with saturated NaCl solution, the mixture was shaken and left standing for 2 h. Subsequently, 100 ml of the solution was transferred to a 500 ml separating funnel. Dilute HCl (1:3, Brand: NICE) was added dropwise until the solution became slightly acidic, with an excess of 5 ml HCl. The solution was then allowed to sit for 30 min for the complete conversion of benzoate to benzoic acid. The resulting solution was washed successively with 50, 40, and 30 ml of chloroform, and the washings were collected in a round-bottom flask. The emulsion obtained was diluted with water and washed until a clear solution was achieved. Chloroform was distilled off at 61.20 °C in a water bath, and the residue was dissolved in a minimal amount of acetone, further diluted to 20 ml with water. The solution was titrated with a standard 0.05 N NaOH solution using phenolphthalein as the indicator. The percentage of sodium benzoate in the Kwatha samples was determined using the titration equation provided in standard protocols [36–39].

2.7.2. HPLC analysis

A 50 mg aliquot of the sample was introduced into a 50 ml standard flask and subsequently brought to a 50 ml volume using the mobile phase. The injection volume applied was 20 µl, and the flow rate was maintained at 1 ml/min. The analytical wavelength for the study was set at 254 nm, and the total run time was configured to be 10 min. The HPLC analysis employed an Agilent Technologies 1200 Infinity Series Column with C18 packing material (4.6 × 250mm × 5 µm).

2.8. Statistical analysis

A completely randomized experimental design was used for testing the concentration (in percentage values) of the chemical preservative, sodium benzoate present as well as for testing the pH and TDS in commercial variants of *Sahacharadi Kwatha*. Three replicates of individually prepared samples were used and the results were provided with mean values and standard deviations. One-way analysis of variance (ANOVA)

Table 1
Manufacturing and expiry dates of the commercial *Sahacharadi Kwatha* samples used.

Sample	Date of manufacture	Date of Expiry
S1	Jun-22	May-24
S2	Sep-22	Aug-25
S3	Aug-22	Jul-25

Table 2
Herbal ingredients and their corresponding proportions employed in the preparation of 10 ml of *Sahacharadi Kwatha*.

Herbal Ingredient	Amount of ingredients in 10 ml			Remarks
	S1	S2	S3	
Zingiber officinale	0.666 gm	3.333 gm	4.000 gm	Reference text used: Ashtanga hridayam chikitsa sthana chapter 21, sloka 56
Cedrus deodara	1.417 gm	6.667 gm	4.000 gm	
Barleria prionitis	–	10.000 gm	–	Preparation as per reference: 1 part each of the three herbs •Sahachara (*Barleria prionitis*), •Suradaru (Devadaru) (*Cedrus deodara*), and •Sunthi (*Zingiber officinale*)
Strobilanthes ciliatus	2.083 gm	–	–	
Nilgirianthus ciliatus	–	–	4.000 gm	
Sida cordifolia	4.167 gm	–	–	

Table 3
Organoleptic characteristic of *Sahacharadi Kwatha* variants available in the market.

Sample	Colour	Odour	Taste
S1	Dark brown	Characteristic odour	Bitter
S2	Light brown	Characteristic odour	Bitter
S3	Light brown	Characteristic odour	Bitter

was performed and the significance of each mean property value was determined ($p < 0.001$) using SPSS software.

3. Results

3.1. Sample collection

Relevant information regarding manufacturing dates and expiry dates of the samples is presented in Table 1. The tests were executed during the period spanning November 2022 to July 2023.

Each sample container prominently displayed informative labels elucidating the herbal components utilized in the formulation of *Sahacharadi Kwatha*. The precise quantities of herbs essential for S1 formulation (10 ml), S2 formulation (15 ml), and S3 formulation (20 ml) were explicitly delineated on their respective containers. Table 2 offers a comprehensive enumeration of the names and quantities of individual herbal ingredients incorporated in the production of samples S1, S2, and S3. The labels on each container explicitly conveyed that the formulation of samples S1, S2, and S3 adhered to the principles outlined in Ashtangahridayam. Table 2 highlighted the shared use of *Zingiber officinale* and *Cedrus deodara* as common herbs in the preparation of *Sahacharadi Kwatha*, while *Sida cordifolia* was exclusively employed in the formulation of S1. Despite the traditional prescription of *Barleria prionitis* in classical Ayurvedic texts, it appears that *Strobilanthes ciliatus* and *Nilgirianthus ciliatus* are substituted in the preparation of S1 and S3, respectively. Additionally, Table 2 revealed significant variations in the quantities of ingredients utilized, despite the common reference in all three commercial preparations. It is noteworthy that none of the sample containers provided information regarding the type and quantity of preservatives incorporated into the formulations (see Table 3).

3.2. Organoleptic characterization

In terms of coloration, Sample S1 exhibited a dark brown hue, whereas both Sample S2 and Sample S3 displayed a lighter brown coloration. All three samples shared a pungent characteristic odour. Despite a consistently bitter taste across all samples, there was a characteristic variation in the intensity of bitterness.

3.3. Physicochemical analysis

The figures provided depict the pH values and Total Dissolved Solids (TDS) content of the samples, with pH levels ranging from 5.2 to 5.99, with TDS content ranging from 6.5 parts per trillion (ppt) to 6.8 ppt. The pH value is a measure of the acidity or alkalinity of a solution. A pH of 7 is considered neutral, while values below 7 indicate acidity, and values above 7 indicate alkalinity. The lower the pH, the more acidic the substance.

3.4. Microbial load analysis

Bacteria were detected in all three samples, with a total plate count below 105 cfu/gm, indicating consistency across the samples. The total plate count is a measure of the number of viable bacteria present in a sample. A count below 10^5 cfu/gm is generally accepted. Furthermore, various classes of fungi were detected at levels less than 10^3 cfu/gm (Table 4). Fungi are ubiquitous in nature and can be found in soil, air, and water. The bacterial contaminants comprised both Gram-negative and Gram-positive species, mainly bacilli with a few cocci. The fungal colonies displayed morphologies typical of *Aspergillus* and *Penicillium* species.

3.5. Phytochemical analysis

The preliminary phytochemical screening results of the samples are summarized in Table 5. The table indicates the presence or absence of various phytochemical constituents in the samples. Flavonoids, phenol, saponins, tannins, carbohydrates, and terpenoids were consistently present across all three samples, while alkaloids and glycosides were absent. Phytochemicals are bioactive compounds found in plants, and their presence can indicate the potential medicinal or nutritional value of the samples. The consistent presence of flavonoids, phenol, saponins, tannins, carbohydrates, and terpenoids across the samples suggests the potential presence of these beneficial compounds. This suggests a uniformity in the presence of these phytochemicals across the samples. Additionally, Table 6 illustrates the percentage variation of phytochemicals in the S1, S2, and S3 samples. The figure shows that sample S2 exhibited the highest phytochemical content, while S1 had the lowest among the three samples. This variation in phytochemical content among the samples is an important finding and may have implications for their potential applications.

3.6. HPTLC profiling

The HPLC fingerprint profiling of the samples revealed differences in the number of bands, peaks, maximum Rf, and area percentage among S1, S2, and S3 samples. At 254 nm S1 exhibited 7 peaks, whereas S2 exhibited 12 and S3 exhibited 13 peaks. When observed at 366 nm, S1,

Table 4
Total Plate Count (TPC) for samples S1, S2 and S3.

Sample	Microbial load (TPC)	
	Bacteria	Fungi
S1	Less than 10^5 cfu/gm	Less than 10^3 cfu/gm
S2	Less than 10^5 cfu/gm	Less than 10^3 cfu/gm
S3	Less than 10^5 cfu/gm	Less than 10^3 cfu/gm

Table 5
Preliminary qualitative phytochemical analysis of S1, S2 and S3.

Phytochemicals	S1	S2	S3
Alkaloids	–	–	–
Flavonoids	+	+	+
Glycosides	–	–	–
Phenol	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Carbohydrate	+	+	+
Terpenoids	+	+	+

Table 6
Quantitative phytochemical analysis of S1, S2 and S3.

Phytochemical	Amount in percentage		
	S1	S2	S3
Flavanoids	0.08	0.04	0.07
Phenol	0.32	0.72	0.56
Saponins	1.1	2.73	2.13
Tannins	0.74	2.08	1.24
Carbohydrate	2.19	2.1	2.37

S2, and S3 presented 9, 13, and 13 peaks, respectively.

Fig. 1a and 1b show the HPTLC peak details of samples S1, S2, and S3 at 254 nm and 366 nm. From Fig. 2, it is clear that Sample S1 lacks Vasicine, while samples S2 and S3 contain this key phytochemical, known for its therapeutic properties in *Sahacharadi Kwatha*. The presence of Vasicine in Samples S2 and S3 highlights the importance of quality control for consistent therapeutic effects. Gallic acid is present in all three samples, indicating a standardized manufacturing process for *Sahacharadi Kwatha*. Beta-sitosterol is consistently found in all samples, suggesting its significant role in the therapeutic effects of the kwatha. Further quantification analysis is needed to determine the exact amount of beta-sitosterol for standardization and understanding its therapeutic potential.

3.7. Preservative load analysis

The titrimetric method was initially employed to determine the quantity of sodium benzoate in the samples. This method involves using a titrant to react with the analyte (sodium benzoate) until the reaction reaches its equivalence point, allowing the determination of the analyte's concentration. The titrimetric results were then compared with the results of High-Performance Liquid Chromatography (HPLC) analysis, which is a highly accurate technique for separating and quantifying components in a mixture. S2 contains higher levels of sodium benzoate compared to S1 and S3. Among the three samples, S3 exhibited the lowest quantity of sodium benzoate. The percentage of sodium benzoate in the analyzed samples ranged from 0.4% to 0.6%. The use of the titrimetric method in conjunction with HPLC analysis provides complementary information about the sodium benzoate content in the samples.

4. Discussion

The study aimed to evaluate the quality parameters of *Sahacharadi Kwatha*, an Ayurvedic formulation commonly used for managing Vata-related diseases. The manufacturing of *Sahacharadi Kwatha* in three different brand samples was guided by the traditional text Ashatangahridayam. However, deviations from standard procedures were observed, indicating alterations made by manufacturers for convenience. These deviations included the addition of *Sida cordifolia* in sample S1 and the substitution of *Barleria prionitis* with alternative herbs in some samples, potentially due to non-availability, seasonal variations, local preferences, or economic considerations. The study employed

Fig. 1a. Chromatogram under UV 254 nm.

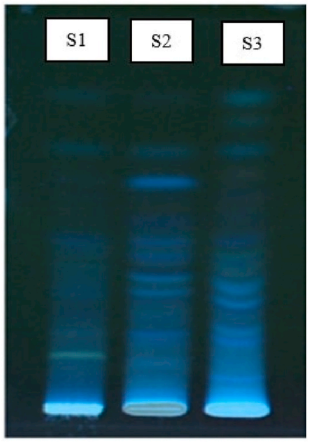

Fig. 1b. Chromatogram under UV 366 nm.

various analytical methods to assess the quality of the samples.

The findings of the present study revealed that, while the manufacturers follow the basic instructions of the standard text books for *Kwatha* preparation they also alter various steps to their convenience. As per standard references, the herbal ingredients recommended for the preparation of *Sahacharadi Kwatha* include *Zingiber officinale, Cedrus deodara*, and *Barleria prionitis*. In sample S1, *Sida cordifolia* is included in addition to the standard herbs. Some manufacturers, instead of using *Barleria prionitis*, uses alternative herbs such as *Nilgirianthus ciliatus* or *Strobilanthes ciliatus*. The reasons for the addition of alternative herbs might be the non-availability of the standard recommended herbs in certain regions, seasonal variations, local preferences, and alignment with regional practices, economical compatibility, or the easy accessibility of the herbs. The use of alternative herbs in Ayurvedic formulations requires careful consideration to maintain overall efficacy and safety. It is essential to ensure that the substituted herbs align with the principles of Ayurveda and contribute positively to the therapeutic outcomes [40, 41]. The standardization of Ayurvedic formulations and the correlation between traditional and modern processing methods are essential for ensuring the quality, safety, and efficacy of Ayurvedic herbal medicines.

Organoleptic analysis, which involves sensory evaluation, revealed characteristic features such as a brown color and bitter taste in all three brands, with brand-specific variations. This analysis highlighted the importance of assessing product characteristics to understand the quality of Ayurvedic formulations. Sensory evaluation methods, in combination with chemical and instrumental analyses, provide a comprehensive approach to assessing the quality and fingerprinting of

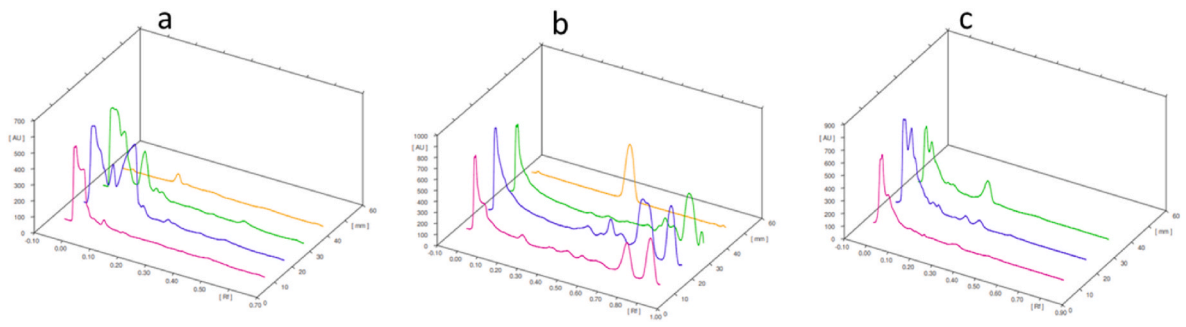

Fig. 2. Overlay chromatogram of all samples along with markers a) Vasicine at 254 nm b) Gallic acid at 254 nm c) Beta sitasterol at 254 nm Color code: Pink - S1; Blue - S2; Green - S3, Yellow - Markers.

Ayurvedic formulations. They play a crucial role in ensuring the safety, efficacy, and standardization of these traditional medicines [42].

Physicochemical analysis provided insights into the acidity, total dissolved solids (TDS) values, and the presence of various phytochemicals in the samples. The slightly acidic pH and variations in TDS values among the brands suggested potential impacts on the medicinal quality of the formulations. The correlation between TDS and specific phytochemicals suggests that the composition of *Sahacharadi Kwatha* is intricately linked to its total dissolved solids. The presence of tannins, alkaloids, phenols, and sterols in higher concentrations in sample S2 likely contributes to its elevated TDS values. The high TDS in sample S2 correlated with the content of specific phytochemicals, such as tannins, alkaloids, phenols, and sterols, which contribute to the pharmacological properties of the formulations. Tannins have antimicrobial properties and are recognized for their role in extending the shelf life of herbal preparations [43,44]. Alkaloids, phenols, and sterols also contribute to the overall therapeutic effects of the formulation, influencing its efficacy in managing Vata-related diseases [45]. TDS is a measure of the combined content of all inorganic and organic substances present in a liquid. In the context of *Sahacharadi Kwatha*, variations in TDS values were observed among the different brands. TDS values can be influenced by factors such as the selection of raw materials, their proportions, and specific processing steps. High TDS in sample S2 is noteworthy.

Microbial contamination in herbal formulations was also evaluated, emphasizing the importance of assessing the microbial load in raw materials to ensure the therapeutic benefits of the formulations. The bacterial contaminants included both gram-negative and gram-positive species, predominantly bacilli with some cocci. The fungal colonies exhibited morphologies characteristic of *Aspergillus* and *Penicillium* species. Microbial contamination in herbal formulations poses a significant health risk and can compromise the therapeutic benefits of these products. Several studies have highlighted the prevalence of microbial contaminants in herbal medicines, emphasizing the importance of assessing the microbial load to ensure product safety and efficacy [46]. A study on the microbial contamination of herbal medicines in Bangladesh revealed the presence of pathogenic bacteria such as *Salmonella, Escherichia coli, Staphylococcus aureus*, and *Shigella* spp. in the products, raising concerns about their safety for consumers [47]. Similarly, a study conducted in Saudi Arabia identified and quantified microbial contaminations in herbal medicines, demonstrating the presence of pathogenic bacteria and fungi above safety limits, which could pose serious health hazards [48]. In our present study, we noted the addition of sodium benzoate as a preservative to enhance shelf life, but the levels exceeded FDA and API prescribed limits, raising concerns about compliance with regulatory standards. Research on the contamination of herbal medicinal products in low-and-middle-income countries (LMICs) identified bacterial contaminants as a prevalent issue. We emphasize the need for robust quality control measures to address microbial contamination in herbal medicines. The addition of preservatives such as sodium benzoate to herbal formulations is a common practice to enhance shelf life. This finding underscores the importance of adhering to regulatory guidelines to ensure the safety and quality of herbal formulations.

High-Performance Thin-Layer Chromatography (HPTLC) profiling indicated quality variations among the brands, supporting HPTLC fingerprinting as a reliable method for assessing the quality of Ayurvedic medicines. For instance, a study focused on the development and validation of an HPTLC method for the determination of gallic acid, catechin, and resveratrol in commercially available polyherbal formulations [49]. This research highlighted the utility of HPTLC in the quality assessment of complex herbal mixtures, supporting its role in ensuring the presence of specific bioactive compounds. Another study on the profiling and determination of phenolic compounds in polyherbal formulations and their comparative evaluation utilized HPTLC analysis for the fingerprinting of phytochemical constituents, demonstrating its effectiveness in assessing the quality and consistency of Ayurvedic products [50]. Several other studies also highlighted the promising approach of combining sensorial and chemical descriptors, such as HPTLC, for a comprehensive evaluation and fingerprinting of the Ayurvedic pharmacological properties of medicinal plants [42]. Vasicine, gallic acid, and B-sitosterol are key phytochemicals found in various Ayurvedic formulations, including Kwathas. These compounds play crucial roles in the therapeutic effects of these formulations, contributing to their antimicrobial, antioxidant, and anti-inflammatory properties. Vasicine is an alkaloid found in Adhatoda vasica, a plant used in Ayurvedic medicine. It exhibits bronchodilatory, respiratory stimulant, and uterine stimulant effects. Vasicine acetate, a derivative of Vasicine, has shown antimycobacterial activity and cytotoxic effects against A549 lung adenocarcinoma cancer cell line [51]. Gallic acid is a phenolic compound that possesses antioxidant, anti-inflammatory, and antimicrobial properties. It is present in various Ayurvedic formulations and contributes to their therapeutic effects [52]. B-sitosterol is a plant sterol that is a significant component in Ayurvedic formulations. It is known for its therapeutic effects and contributes to the pharmacological benefits associated with traditional Ayurvedic medicine [53].The use of HPTLC profiling has been well-documented in the literature as a reliable method for assessing the quality of Ayurvedic medicines. Its application in the development of marker compound quantification, fingerprinting of phytochemical constituents, and comprehensive evaluation of herbal formulations underscores its significance in ensuring the safety, efficacy, and consistency of Ayurvedic products. Ongoing research in this area continues to support the utility of HPTLC as a valuable tool for the quality assessment of herbal medicines.

The results of our present study provided additional insights into the standardization of Ayurvedic formulations, the importance of quality control, and the correlation between traditional and modern processing methods in the Ayurvedic industry. These findings support the significance of ongoing research and quality assessment in the field of Ayurvedic medicine.

5. Conclusion

The present study has unveiled considerable differences in quality parameters and preservative content among the diverse market formulations of *Sahacharadi Kwatha*. The findings substantiate the notion of inconsistent standards prevailing in the Ayurvedic medicine market, highlighting a crucial demand for the establishment of rigorous regulatory frameworks and standardization procedures for Ayurvedic formulations. The observed variations emphasize the need for a comprehensive approach to validation processes, underscoring the urgency in addressing the prevalent unregulated practices. Establishing standardized protocols not only ensures the reliability of Ayurvedic medicines but also plays a pivotal role in strengthening consumer trust, consequently fostering increased acceptability of these formulations. Collaborative efforts from regulatory bodies, practitioners, and stakeholders are essential to institute robust quality control measures, ultimately promoting a more secure and standardized landscape for Ayurvedic healthcare solutions. The standardization of herbal formulations is essential to assess the quality of drugs based on the concentration of their active principles, and it requires a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulations. This can be achieved through the evaluation and analysis of herbal products using sophisticated methods and technologies to meet the growing demand for standardized, therapeutically effective Ayurvedic formulations. This, in turn, contributes significantly to the overarching advancement and expansion of the Ayurveda industry. Therefore, the study underscores the urgency of collaborative efforts from regulatory bodies, practitioners, and stakeholders to institute robust quality control measures, ultimately promoting a more secure and standardized landscape for Ayurvedic healthcare solutions.

Declaration of generative AI in scientific writing

None.

Author contributions

Both the authords contributed to data acquisition and manuscript preparation. Sangeetha Gopal: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing - Original Draft, Visualization, Project Administration, and Funding Acquisition. Leon Ittiachen: Validation, Resources, Writing - Review & Editing, Supervision, and Funding Acquisition.

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Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Sangeetha Gopal reports financial support was provided by Kerala Development and Innovation Strategy Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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