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Original Article

# Comparative Pharmaceutical and Analytical Evaluation of Shoolaharana Yoga: Bhavita Formulation Versus Hydroalcoholic Extract

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## ABSTRACT

**Background:** Shoolaharana Yoga, described in Rasendra Sara Saṃgraha, is a classical Herbo-mineral formulation indicated for various types of pain. Ayurvedic pharmaceutical processes such as Shodhana (Purification) and Bhavana (Trituration) are traditionally employed to enhance safety, bioavailability, and therapeutic potential. In the present era, integration of classical processing with modern extraction techniques warrants systematic analytical evaluation. **Objectives:** The present study aimed to evaluate pharmaceutical and analytical parameters of Shoolaharana Yoga processed with Shoolprashamana Mahakashaya *Bhavana* (SHYB) and its Hydroalcoholic Extract (SHYBE). **Materials and Methods:** Shoolaharana Yoga was prepared after Shodhana of Gandhaka, Hingu, and Kuchala, followed by three Bhavans using decoction of Shoolprashamana Mahakashaya as described in Charaka Samhita *Sutra Sthana* 4/45. The Bhavita formulation (SHYB) was subjected to hydroalcoholic extraction to obtain SHYBE. Both samples were evaluated for various analytical parameters as per the protocols prescribed by the Ministry of AYUSH. **Results:** The extract (SHYBE) showed markedly higher water- and alcohol-soluble extractive values, indicating superior extraction efficiency compared to the base formulation. Both samples were found to be within the permissible limits for heavy metals and microbial load as per Ayurvedic Pharmacopoeia. HPTLC analysis demonstrated an increased number of spots across multiple wavelengths in SHYBE, reflecting enhanced phytochemical complexity following extraction. **Conclusion:** The study establishes that the hydroalcoholic extraction method yields better phytochemical extraction and a richer chromatographic profile compared to the conventional method. This indicates that the extraction technique significantly influences the pharmaceutical quality and standardization of the formulation.

**Keywords:** Analytical study, Bhavana, Hydroalcoholic extract, Shool, Shoolharan Yoga, Shoolprashamana Mahakashaya.

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## INTRODUCTION

Pain disorders, particularly spasmodic and visceral pain, remain a major global health burden and continue to drive extensive use of both conventional antispasmodics and traditional medicines. However, concerns regarding adverse effects of commonly used drugs such as NSAIDs and anticholinergics have renewed interest in traditional polyherbal and Herbo-mineral formulations with multimodal mechanisms of action (Harirforoosh *et al.*, 2013; Cohen *et al.*, 2021). *Shoolaharana Yoga*, described in Rasendra Sara Saṃgraha, is a classical Ayurvedic Herbo-mineral formulation indicated in various types of Shoola (colicky and spasmodic pain). The formulation comprises ingredients possessing documented Deepana (Digestive stimulant), Pachana (Digestive), Vatanulomana (Carminative) and Shoolaghna (Analgesic) properties (Tripathi, 2014). Despite long-standing traditional use and emerging pharmacological evidence supporting its antispasmodic potential, a critical gap persists in its comprehensive pharmaceutical and analytical standardization.

One of the major challenges in the global acceptance of traditional formulations is the lack of robust physicochemical characterization, reproducible extractive profiling, safety validation, and chromatographic fingerprinting. Polyherbal-Herbo-mineral preparations are particularly susceptible to variability due to differences in raw material quality, processing techniques (such as Bhavana), and extraction methodologies. Without rigorous analytical benchmarks, batch-to-batch consistency, safety assurance, and regulatory acceptance remain limited.

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*Bhavana* (levigating with specific liquid media) is a classical pharmaceutical process believed to enhance bioavailability and therapeutic potency through physicochemical modification and improved integration of constituents. Although prior studies have suggested that processing *Shoolaharana Yoga* with Shoolprashamana Mahakashaya Bhavana enhances its pharmacological activity, there remains insufficient analytical evidence elucidating the physicochemical transformations induced by *Bhavana* and subsequent extraction procedures (Sharma, 2004).

Furthermore, hydroalcoholic extraction offers a scientifically rational approach for selectively concentrating both polar and semi-polar phytoconstituents, potentially altering extractive values, chromatographic profiles, and overall chemical density of the formulation. However, systematic analytical comparison between the *Bhavita* formulation and its hydroalcoholic extract has not been adequately documented.

Therefore, the present study was designed to undertake a comparative pharmaceutical and analytical evaluation of *Shoolaharana Yoga* processed with *Shoolprashamana Mahakashaya Bhavana* and its hydroalcoholic extract. The investigation focused on physicochemical parameters, extractive values, heavy metal and microbial analysis, and chromatographic fingerprinting, with the objective of establishing reproducible quality control markers and generating foundational data for future pharmacological and clinical investigations.

By addressing the standardization gap, the present study aims to contribute toward the scientific validation, quality assurance, and regulatory strengthening of a classical Ayurvedic formulation within the framework of contemporary pharmaceutical sciences. The study was undertaken to carry out a comparative analytical evaluation of *Shoolaharana Yoga* processed with *Shoolaprashamana Mahakashaya Bhavana* (SHYB) and its hydroalcoholic extract (SHYBE) in order to assess the impact of Bhavana and extraction on physicochemical characteristics, and phytochemical profile

## OBJECTIVES

- To prepare Shoolaharana Yoga following classical pharmaceutical procedures, including Shodhana of selected ingredients and Bhavana using Shoolprashamana Mahakashaya decoction.
- To prepare the Hydroalcoholic Extract (SHYBE) of Shoolprashamana Mahakashaya Bhavita Shoolaharana Yoga using a standardized Soxhlet extraction method.
- To evaluate and compare the organoleptic and physicochemical parameters of SHYB and SHYBE, including pH, moisture content, and extractive values.
- To assess the safety profile of both formulations through heavy metal analysis and microbial limit testing as per AYUSH guidelines.
- To develop and compare chromatographic fingerprints of SHYB and SHYBE using High-Performance Thin Layer Chromatography (HPTLC) for qualitative phytochemical profiling.

## MATERIALS AND METHODS

### Study Design

The present work is a comparative pharmaceutical and analytical study designed to evaluate Shoolaharana Yoga processed with Shoolprashamana Mahakashaya Bhavana (SHYB) and its hydroalcoholic Extract (SHYBE). The study was conducted in accordance with standard protocols prescribed for the analysis of Ayurvedic formulations.

## ETHICAL APPROVAL

Ethical approval was not required for the present study, as it involved only pharmaceutical and analytical evaluation of the formulation and did not include any human or animal experimentation (Table 1).

### Raw Materials and Authentication

According to recent botanical surveys,*Gandeera* exhibits taxonomic ambiguity; therefore, excluded to maintain reproducibility. All raw drugs were procured from the local market of Haridwar, Uttarakhand, and were authenticated as per API protocols by expert of P.G. Department of *Dravyaguna* & P.G. Department of *Rasashastra* & *Bhaishajya Kalpana*, Rishikul Campus, Haridwar prior to use.

### Pharmaceutical Processing

#### *Shodhana of Ingredients*

Classical *Shodhana* procedures were carried out for Gandhaka, Hingu, and Kuchala prior to formulation.

Percentage weight loss during *Shodhana* was calculated to assess the extent of impurity removal.

### Preparation of Shoolaharana Yoga with Bhavana (SHYB)

All Shuddha ingredients of Shoolaharana Yoga were individually powdered, sieved through sieve no. 80, and mixed in equal proportions. Shoolprashamana Mahakashaya Kwatha was prepared by boiling the coarse powder of *Mahakashaya* drugs with eight times water and reducing it to one-eighth of the initial volume (Sharangdhar, 2004).

Three successive Bhavanas were administered to Shoolaharana Yoga using freshly prepared *Kwatha*, with complete drying

between each trituration. The final Bhavita formulation (SHYB) was dried and stored in airtight containers for further analysis.

## Preparation of Hydroalcoholic Extract (SHYBE)

SHYB was subjected to hydroalcoholic extraction using a Soxhlet apparatus. A solvent system comprising 40% distilled water and 60% ethanol was employed. Continuous extraction was carried out for 8 hr. The extract was filtered and concentrated using distillation followed by rotary evaporation at 29°C. The dried extract (SHYBE) was collected, weighed to calculate percentage yield, and stored in airtight containers.

## Analytical Evaluation

Powders of Both SHYB and SHYBE were analysed at Vasu Research Centre, Gujarat. Analytical parameters were assessed as per the "Protocol for Testing of Ayurvedic, Siddha and Unani Medicines" published by the Ministry of AYUSH, Government of India, and the Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H). Organoleptic characteristics such as colour, odour, taste, and appearance were assessed along with physicochemical parameters including pH and extractive values. Heavy metal analysis for Lead, Cadmium, Arsenic, and Mercury, as well as microbial limit tests, were also performed to ensure the safety and standardization of the formulation.

## HPTLC Fingerprinting

### *Preparation of Test Solution*

Accurately weighed 0.5 g of each sample was dissolved in 10 mL of methanol and sonicated for 30 min. The solution was centrifuged for 10 min, and the supernatant was collected and used as the test solution.

## Chromatographic Conditions

- **Stationary phase:** Silica gel 60 $F_{254}$ precoated aluminium plates.
- **Application:** CAMAG Lino mat 5.
- **Mobile phase:** Toluene: Ethyl acetate: Formic acid (7:2:1 v/v).
- **Chamber saturation:** 30 min.
- **Sample volume:** 8 µL.
- **Development:** CAMAG Twin Trough Chamber.

## Derivatization and Detection

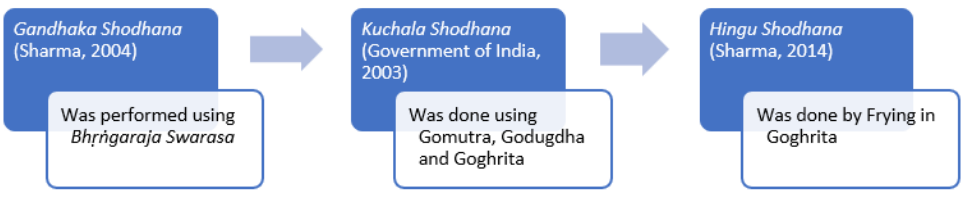
Plates were visualized at 254 nm and 366 nm prior to derivatization. Post-derivatization was performed using anisaldehyde-sulphuric acid reagent, followed by heating at 100±5°C for 3 min. Final visualization was carried out at 540 nm. $R_f$ values were recorded and chromatographic profiles of SHYB and SHYBE were compared.

## Heavy Metal Analysis

Heavy metal analysis was performed using Perkin Elmer AAS-200 instrument. As per the protocol, sample digestion was carried out by multi-acid digestion system for Lead (Pb), Cadmium (Cd), Mercury (Hg) and Arsenic (As). After completion of the digestion process, the filtered samples were analysed by Atomic Absorption Spectrometer (AAS). However, being volatile, mercury (Hg) and Arsenic (As) were digested using nitric acid-hydrochloric acid-potassium permanganate system before analysis. The Mercury Vapour Atomization (MVA) and Hybrid Vapour Generation (HVG) attachments were utilised for AAS analysis of Hg and As, respectively. The standards of Pb, Cd, As and Hg, were purchased from Merck, Germany and utilised for development of the respective calibration curves for these metals (Mukhi *et al.*, 2017).

**Table 1: Formulation composition.**

| Sl. No. | Component | Details |
|---|---|---|
| 1. | Formulation Name | Shoolaharana Yoga |
| 2. | Main Ingredients | *Haritaki, Trikatu (Shunthi, Maricha, Pippali), Shudha Kuchala, Hingu, Saindhava Lavana, Shudha Gandhaka* (Tripathi.I., 2014) |
| 3. | *Bhavana* Dravya | Shoolaprashamana Mahakashaya |
| 4. | Included Drugs in *Bhavana* Media | *Pippali, Pippalimula, Chavya, Citraka, Shunthi, Maricha, Ajamoda, Ajaji, Ajagandha, Gandeera* (Pandey, K., & Chaturvedi, G. 2009) |



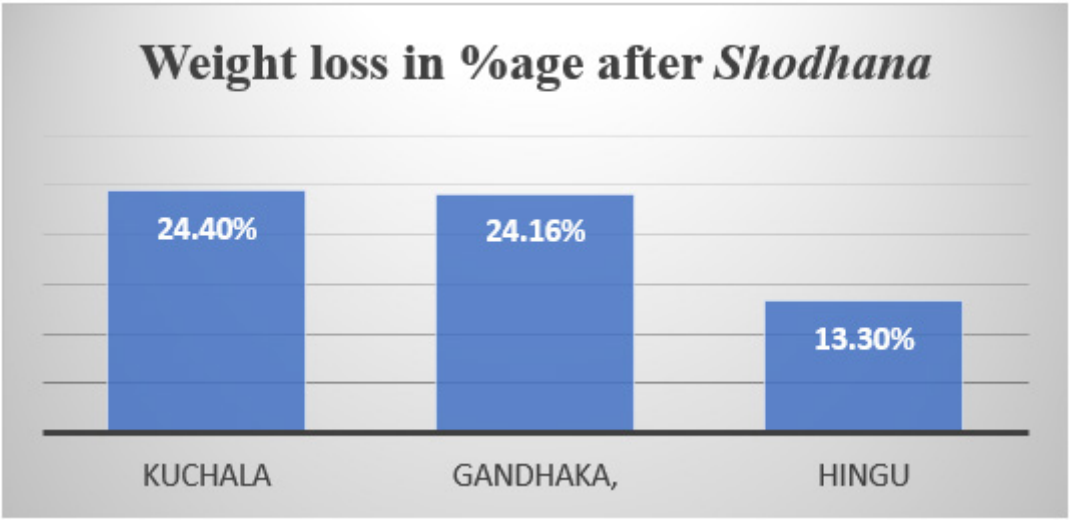



**Figure 1:** Yield after *Shodhana*.

**Table 2: Organoleptic characteristics and Physico-chemical parameters of SHYB and SHYBE.**

| Parameters | SHYB | SHYBE |
|---|---|---|
| *Rupa* (Colour) | Brown Powder | Dark Brown Resinous powder |
| *Gandha* (Odour) | Characteristic smell of *Hingu* | Characteristic smell of *Hingu* |
| *Rasa* (Taste) | Slightly bitter | Bitter |
| pH (% aq. sol) | 4.67 | 3.55 |
| Loss on drying (% w/w) | 10.19% | 7.19% |
| Ash Value (% w/w) | 15.36% | 32.80% |
| Acid Insoluble Ash (% w/w) | 10.22% | 21.77% |
| Water Soluble Extractive Value (% w/w) | 37.74% | 76.26% |
| Alcohol Soluble Extractive Value (% w/w) | 30.06% | 72.34 |

### Microbial Load assessment

Microbial analysis was carried out as per standard procedure mentioned in API. It included total bacterial count, total fungal count, presence of pathogens like *Escherichia coli, Salmonella ebony, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Baragi *et al.*, 2011).

## RESULTS

### Observations during *Shodhana*

Classical *Shodhana* procedures resulted in a measurable reduction in the weight of selected ingredients, indicating effective removal of impurities. The average percentage weight loss observed was 24.4% in Kuchala, 24.16% in Gandhaka, and 13.3% in Hingu. These reductions substantiate the role of Shodhana in detoxification and pharmaceutical refinement of herbo-mineral drugs (Figure 1).

### Yield of Hydroalcoholic Extract

Hydroalcoholic extraction of Shoolprashamana Mahakashaya Bhavita Shoolaharana Yoga (SHYB) yielded 26.9% extract (SHYBE), reflecting effective extraction and concentration of soluble phytoconstituents through the selected solvent system.

### Organoleptic Characteristics

As presented in Table 2, both SHYB and SHYBE exhibited comparable organoleptic properties with respect to odour and appearance, except for variations in colour and taste. SHYB was light brown in colour, whereas SHYBE appeared dark brown, possibly due to concentration of phytochemicals following extraction. The characteristic odour of Hingu was retained in both formulations, indicating stability of volatile constituents during pharmaceutical processing and extraction.

### Physicochemical Parameters

As presented in Table 2, both formulations exhibited an acidic pH, consistent with the inherent nature of their constituent drugs. SHYBE showed a comparatively lower pH than SHYB, indicating a higher concentration of acidic components following hydroalcoholic extraction. Loss on drying was lower in SHYBE, suggesting reduced moisture content and improved formulation stability.

The ash value and acid-insoluble ash were higher in SHYBE compared to SHYB, reflecting an increased concentration of inorganic and mineral constituents as a consequence of the extraction process. A marked enhancement in extractive values was also observed, with water-soluble extractive increasing from 37.74% to 76.26% and alcohol-soluble extractive from 30.06% to 72.34%. The near doubling of both extractive values in SHYBE

indicates superior solubilization and enrichment of bioactive phytoconstituents.

## Heavy Metal Analysis

As shown in Table 3, the concentrations of lead, arsenic, cadmium, and mercury in both SHYB and SHYBE were found to be well within the permissible limits prescribed in the Ayurvedic Formulary of India (AFI). Lead was detected at 2.69 ppm in SHYB, while it was not detected in SHYBE. Mercury levels were minimal in both formulations, measuring 0.37 ppm in SHYB and 0.11 ppm in SHYBE, whereas arsenic and cadmium were not detected in either sample. These findings confirm the safety and acceptable quality of the formulations and substantiate the effectiveness of classical *Shodhana* procedures employed during pharmaceutical processing.

## Microbial Load Assessment

As presented in Table 3, microbial evaluation revealed that the total bacterial count as well as total yeast and mould count in both SHYB and SHYBE were either within permissible limits or below detectable levels. The total plate count was 165 cfu/g for SHYB and 10 cfu/g for SHYBE, both well below the prescribed

**Table 3: Heavy metal analysis and Microbial limit test of SHYB and SHYBE.**

| Heavy metal analysis of SHYB and SHYBE | | | |
|---|---|---|---|
| **Heavy Metals** | **Permissible limit (API)** | **SHYB** | **SHYBE** |
| Lead | NMT 10ppm | 2.69PPM | Not detected |
| Arsenic | NMT 3ppm | Not detected | Not detected |
| Cadmium | NMT 0.3ppm | Not detected | Not detected |
| Mercury | NMT 1ppm | 0.37ppm | 0.11ppm |
| **Microbial limit test of SHYB and SHYBE** | | | |
| **Test (cfu/gm)** | **Permissible limit (API)** | **SHYB** | **SHYBE** |
| Total plate count | NMT $10^5$ cfu/g | 165cfu/g | 10 cfu/g |
| Total Yeast and Mould Count | NMT $10^3$ cfu/g | Absent | Absent |
| *Escherichia coli* | Absent | Absent | Absent |
| *Pseudomonas aeruginosa* | Absent | Absent | Absent |
| *Staphylococcus aureus* | Absent | Absent | Absent |
| *Salmonella* Spp. | Absent | Absent | Absent |

**Table 4: Chromatographic profiles of SHYB and SHYBE.**

| At 256 nm | | | At 366 nm | | At 540 nm | |
|---|---|---|---|---|---|---|
| **Spot no.** | **SHYB (Track 1)** | **SHYBE (Track 2)** | **SHYB (Track 1)** | **SHYBE (Track 2)** | **SHYB (Track 1)** | **SHYBE (Track 2)** |
| 1 | -- | 0.10 | 0.17 | 0.17 | -- | 0.17 |
| 2 | 0.17 | 0.17 | 0.28 | 0.28 | 0.20 | 0.20 |
| 3 | 0.28 | 0.28 | 0.35 | 0.35 | 0.28 | 0.28 |
| 4 | 0.35 | 0.35 | -- | 0.40 | -- | 0.32 |
| 5 | 0.44 | 0.44 | 0.44 | 0.44 | 0.35 | 0.35 |
| 6 | 0.49 | 0.49 | 0.49 | 0.49 | 0.38 | 0.37 |
| 7 | 0.57 | 0.57 | 0.57 | 0.57 | 0.40 | 0.40 |
| 8 | 0.65 | 0.65 | 0.65 | 0.65 | 0.49 | 0.49 |
| 9 | 0.71 | 0.71 | 0.71 | 0.71 | -- | 0.53 |
| 10 | -- | 0.74 | 0.74 | 0.74 | 0.57 | 0.57 |
| 11. | -- | -- | 0.81 | 0.81 | 0.65 | 0.65 |
| 12. | -- | -- | -- | -- | 0.71 | 0.71 |
| 13. | -- | -- | -- | -- | 0.77 | 0.76 |
| 14 | -- | -- | -- | -- | -- | 0.79 |
| 15. | -- | -- | -- | -- | 0.81 | 0.81 |

limits. Pathogenic microorganisms, including *Escherichia coli, Staphylococcus aureus, Salmonella* spp., and *Pseudomonas aeruginosa*, were absent in both formulations. These findings confirm the microbiological safety of the formulations and their suitability for internal administration.

HPTLC Fingerprinting AnalysisHPTLC fingerprinting analysis demonstrated qualitative differences between SHYB and SHYBE, indicating variation in phytochemical composition following hydroalcoholic extraction. The chromatographic profiles were recorded at 254 nm, 366 nm, and 540 nm (post-derivatization), and the corresponding $R_f$ values are presented in Table 4.

At 254 nm, SHYB exhibited eight distinct spots, whereas SHYBE showed ten spots, including additional bands at lower and higher $R_f$ values, suggesting the presence of supplementary UV-active constituents in the extract. At 366 nm, SHYB displayed nine spots, while SHYBE revealed ten spots with additional fluorescent bands, indicating enhanced detection of phytoconstituents with conjugated or fluorescent properties. Post-derivatization at 540 nm further accentuated these differences, with SHYB showing eleven spots compared to fifteen distinct spots in SHYBE.

The increased number of resolved spots in SHYBE across all detection wavelengths reflects greater phytochemical complexity and improved extractability of bioactive constituents achieved through hydroalcoholic extraction. These distinct and reproducible HPTLC fingerprints may serve as characteristic markers for quality control and standardization of the formulations (Figures 2 and 3).

## DISCUSSION

The present study was designed to compare the pharmaceutical and analytical profiles of Shoolaharana Yoga processed with Shoolprashamana Mahakashaya Bhavana (SHYB) and its hydroalcoholic extract (SHYBE). As the investigation was limited to analytical.

Classical Shodhana of Gandhaka, Hingu, and Kuchala resulted in appreciable material loss, indicating removal of extraneous and potentially undesirable components. This observation aligns with the classical objective of Shodhana in herbo-mineral formulations, which aims to reduce toxicity and enhance pharmaceutical suitability (Acharya, 2014). The absence of excessive heavy metal content in both SHYB and SHYBE further substantiates the effectiveness of these purification procedures and supports their continued relevance in contemporary formulation practices.

Processing Shoolaharana Yoga with Shoolprashamana Mahakashaya decoction through repeated Bhavana represents a classical strategy to enhance formulation quality. Bhavana facilitates intimate contact between the base formulation and the bioactive principles of the liquid medium, potentially improving uniform distribution and physicochemical integration of constituents. The preservation of characteristic Hingu odour in SHYB indicates that volatile components were not adversely affected during trituration, suggesting pharmaceutical stability of the Bhavita formulation.

Previous studies have suggested that Bhavana can bring alterations in the physicochemical and phytochemical characteristics of formulations (Mitra *et al.*, 2010); however, the extent of these changes and their influence on extractive yield and phytochemical concentration remain inadequately explored.

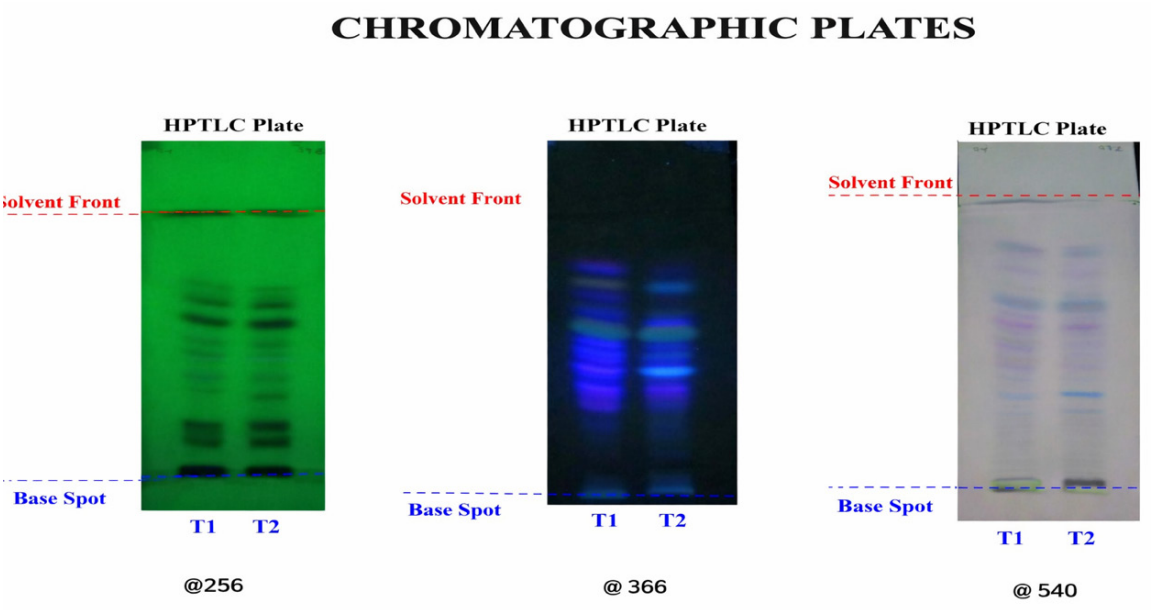



**Figure 2:** HPTLC chromatographic plate of SHYB (T1) and SHYBE (T2) at 256 nm, 366 nm and 540 nm. Note: Track 1= SHYB, Track 2= SHYBE.

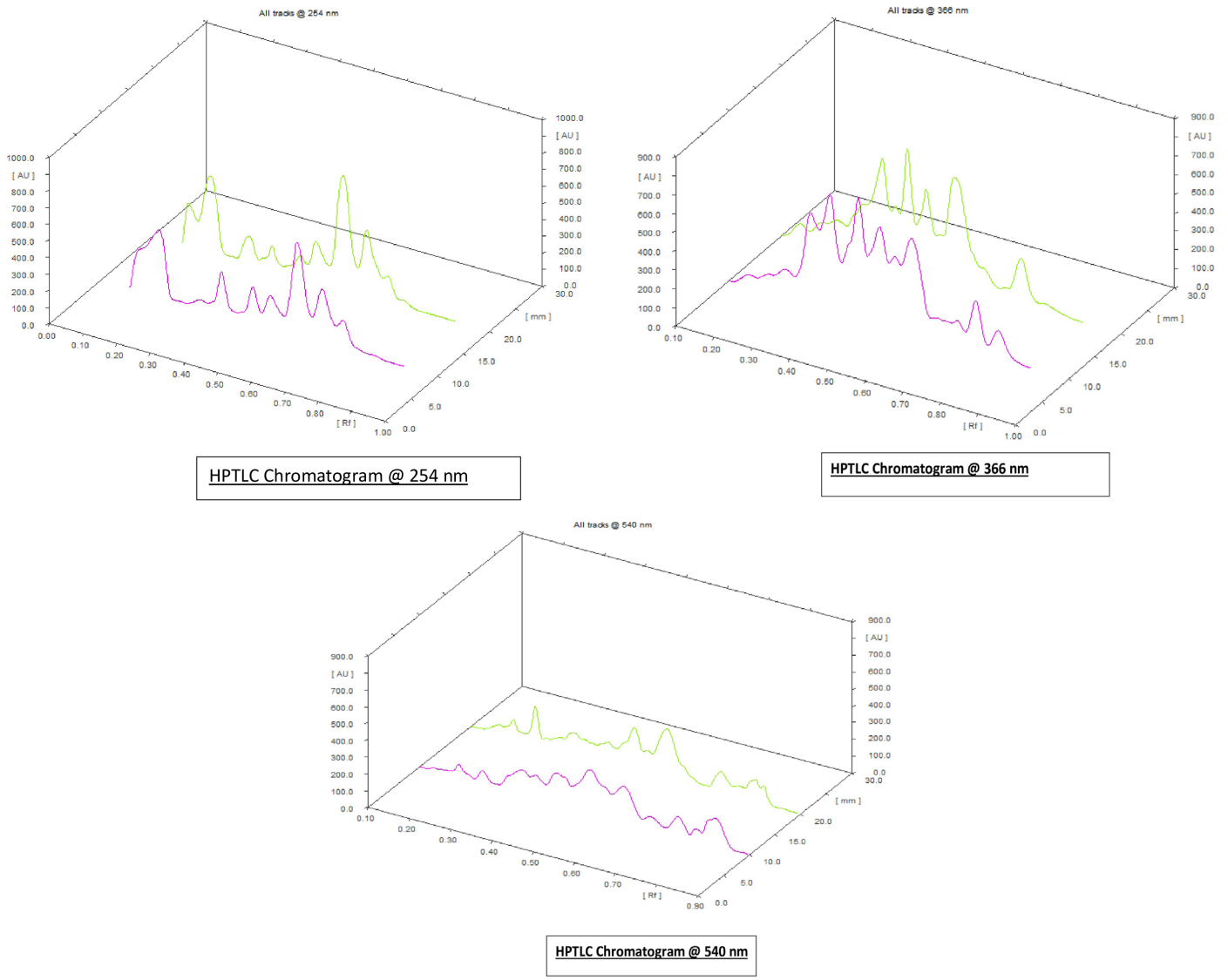



**Figure 3:** Comparative 3D Spectral Intensity Plots at 256, 366, and 540 nm. Note: Pink graph: SHYB, Green graph: SHYBE.

Furthermore, hydroalcoholic extraction is widely employed for efficient isolation and concentration of phytoconstituents (Pandey *et al*., 2021), yet limited evidence is available regarding its application following Bhavana processing and the resultant changes in the analytical profile of the formulation. In this context, the present study was undertaken to evaluate the impact of *Shoolaprashamana Mahakashaya Bhavana* on *Shoolaharana Yoga* and to comparatively assess its hydroalcoholic extract in order to understand how traditional Bhavana processing and subsequent extraction influence the pharmaceutical characteristics, physicochemical properties, and phytochemical profile of the formulation.

Hydroalcoholic extraction of SHYB produced a formulation with markedly higher extractive values compared to the Bhavita powder. This enhancement reflects improved solubilization and concentration of phytoconstituents through the selected solvent system, which is capable of extracting both polar and moderately non-polar compounds. From an analytical perspective, this indicates that extraction yields a more concentrated representation of the formulation's chemical constituents, a property desirable for standardization and reproducibility.

The acidic pH observed in both SHYB and SHYBE is consistent with the nature of constituent drugs and suggests chemical stability of the formulations. The darker colour of SHYBE compared to SHYB may be attributed to increased concentration of extracted constituents, a common characteristic of extract-based preparations.

HPTLC fingerprinting revealed clear qualitative differences between SHYB and SHYBE. The greater number of spots observed in SHYBE across multiple wavelengths indicates enhanced phytochemical expression following hydroalcoholic extraction.

This suggests that certain constituents present in the Bhavita formulation may be present in lower concentrations or remain analytically less prominent unless subjected to extraction. The richer chromatographic profile of SHYBE thus provides a more comprehensive chemical fingerprint, which is advantageous for quality control and batch-to-batch consistency.

From a pharmaceutical standpoint, the findings indicate that while Bhavana contributes to qualitative enhancement and stability of the formulation, hydroalcoholic extraction further concentrates and expresses its phytochemical constituents. The study does not infer therapeutic superiority; however, it establishes that SHYBE is analytically more concentrated than SHYB. These observations provide a rational basis for selecting appropriate dosage forms in future pharmacological and clinical investigations.

## CONCLUSION

The present study establishes that pharmaceutical processing significantly influences the analytical profile of *Shoolaharana Yoga*. *Bhavana* with *Shoolprashamana Mahakashaya* resulted in a stable classical formulation, while subsequent hydroalcoholic extraction yielded a more analytically concentrated product with enhanced extractive values and a richer HPTLC fingerprint. Both formulations complied with prescribed safety parameters. Within the scope of analytical evaluation, SHYBE was found to be pharmaceutically more concentrated than SHYB. Further pharmacological and clinical studies are required to determine the therapeutic implications of these findings.

## LIMITATIONS AND FUTURE SCOPE

The present study is limited to pharmaceutical processing and analytical evaluation of Shoolaharana Yoga processed with Shoolprashamana Mahakashaya Bhavana and its hydroalcoholic extract. Pharmacological, toxicological, and clinical assessments were beyond the scope of the current investigation and therefore no conclusions regarding therapeutic efficacy or clinical superiority can be drawn. Additionally, the study employed qualitative chromatographic profiling without quantification of individual phytoconstituents.

Future research should focus on pharmacological validation of both formulations using appropriate *in vitro* and *in vivo* models to correlate analytical enrichment with biological activity. Standardization through marker-based quantification, bioavailability studies, and stability assessment is also warranted. Well-designed clinical studies may further elucidate the therapeutic relevance of extract-based dosage forms of classical Ayurvedic formulations. Such investigations would strengthen evidence-based integration of traditional pharmaceutical processes with contemporary analytical approaches.

## ACKNOWLEDGEMENT

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## ABBREVIATIONS

**AYUSH:** Ayurveda, Yoga, Unani, Siddha and Homeopathy; **HPTLC:** High Performance Thin Layer Chromatography; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **API:** Ayurvedic Pharmacopeia of India; **AFI:** Ayurvedic Formulary of India.

## CONFLICT OF INTEREST

The author declared that there is no conflict of interest.

## SUMMARY

The present study was undertaken to comparatively evaluate the pharmaceutical processing and analytical characteristics of *Shoolaharana Yoga* processed with *Shoolprashamana Mahakashaya Bhavana* (SHYB) and its hydroalcoholic extract (SHYBE). *Shoolaharana Yoga*, a classical herbo-mineral Ayurvedic formulation indicated in various painful conditions, was prepared following authentic classical procedures including *Shodhana* of *Gandhaka, Hingu*, and *Kuchala*, followed by three successive *Bhavanas* using freshly prepared *Shoolprashamana Mahakashaya Kwatha*. The *Bhavita* formulation (SHYB) was subsequently subjected to hydroalcoholic Soxhlet extraction to obtain SHYBE. Comparative analytical evaluation demonstrated notable physicochemical differences between the two formulations. SHYBE exhibited markedly higher water-soluble and alcohol-soluble extractive values, lower loss on drying, and comparatively higher ash values, indicating enhanced extraction and concentration of phytoconstituents and mineral components. Organoleptic examination revealed darker colour and increased bitterness in SHYBE, reflecting greater chemical concentration following extraction. Heavy metal analysis and microbial limit testing showed that both formulations were within permissible AYUSH limits, confirming their pharmaceutical safety and quality. HPTLC fingerprinting further revealed a greater number of resolved spots in SHYBE across 254 nm, 366 nm, and 540 nm wavelengths, suggesting richer phytochemical complexity and improved analytical expression of constituents after hydroalcoholic extraction. Overall, the study establishes that while *Bhavana* contributes to pharmaceutical refinement and stability of the classical formulation, hydroalcoholic extraction produces a more analytically concentrated preparation with enhanced chromatographic profiling. These findings provide important baseline data for standardization, quality control, and future pharmacological and clinical investigations of *Shoolaharana Yoga*.

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