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ANALYTICAL EVALUATION AND STANDARDIZATION OF SHANKHANJANA

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ABSTRACT
Shankhanjana is a classical Ayurvedic ophthalmic formulation traditionally used in the management of various Netra rogas. The formulation belongs to the Anjana category of ocular drug delivery and is primarily indicated for conditions involving Kapha-dominant pathologies of the eye, owing to its Katu rasa, Sheeta virya, and Kapha-Vata shamaka properties. In the present era, standardization and analytical evaluation of classical formulations are essential to ensure quality, safety, reproducibility, and scientific validation. Hence, the present study aimed to evaluate the physicochemical parameters and HPTLC fingerprint profile of Shankanjana. The formulation was assessed for organoleptic characters, physicochemical properties including alcohol and water soluble extractives and pH, and HPTLC profiling using alcoholic extract with Toluene: Ethyl acetate (9:1) as solvent system. The sample was white in color with pungent odor and tingling and burning sensation on tongue. The pH was found to be 7.5, indicating a near-neutral to mildly alkaline nature suitable for ocular application. Densitometric scanning at 254 nm and 366 nm revealed multiple peaks with distinct Rf values, confirming the presence of diverse phytochemical constituents. The study highlights the importance of physicochemical standardization in establishing quality control parameters for Shankanjana and provides a baseline chromatographic fingerprint for future authentication and quality assurance of this classical ophthalmic preparation.

KEYWORDS: *Ayurveda, Shankhanjana, Anjana, Netra roga, HPTLC.*

INTRODUCTION

Shankhanjana is a classical Ayurvedic formulation that belongs to the Anjana category of ocular preparations. Anjana, or collyrium, represents one of the most important ocular drug delivery systems described in ancient Ayurvedic texts and is administered directly into the eye for the management of a wide range of Netra rogas. Among the various types of Anjana preparations, Shankhanjana holds a significant place due to its broad therapeutic applicability and its characteristic pharmacological properties.

The formulation predominantly contains ingredients with Katu rasa, sheeta virya, and Kapha-Vata shamaka actions, which are therapeutically beneficial in conditions involving excessive secretions, inflammation, microbial activity. The white color, pungent odor, and tingling sensation on contact are characteristic organoleptic features that reflect its active constituents.

In the present era, there is a growing need to subject classical Ayurvedic preparations to rigorous scientific evaluation to ensure their quality, safety, and therapeutic consistency. Standardization of Ayurvedic formulations using modern analytical techniques such as physicochemical evaluation and High Performance Thin Layer Chromatography (HPTLC) is essential for establishing reliable quality control parameters. Such analytical validation not only supports traditional claims but also contributes to the scientific acceptability of classical preparations in contemporary clinical and research settings.

Despite the clinical importance of Shankhanjana, systematic analytical evaluation of this formulation remains limited in the available literature. Therefore, the present study has been undertaken to evaluate the physicochemical and HPTLC parameters of Shankhanjana, to establish a reliable analytical fingerprint that can serve as a standard reference for quality control and future research.

AIM AND OBJECTIVES

To carry out physicochemical analysis and HPTLC fingerprint profiling of Shankhanjana.

MATERIALS AND METHODS

Pharmaceutical Study: Pharmaceutical study started from collection of genuine raw materials, followed by its pre-processing and finally conversion to the product *Shankhanjana*.

1. ORGANOLEPTIC EVALUATION

Organoleptic evaluation of Shankhanjana was carried out using sensory characterization methods employing the senses of vision, smell, and taste. The following parameters were assessed and documented:

- Color: White – Indicates the characteristic appearance of the formulation, reflecting the nature of its mineral and herbal constituents.
- Odor: Pungent – A sharp and penetrating odor suggestive of the presence of volatile and aromatic compounds with therapeutic significance.
- Taste: Tingling and burning sensation on the tongue – Characteristic of compounds with Katu rasa and Ushna virya, in alignment with its Kapha-Vata shamaka properties.

These organoleptic characters collectively confirm the identity and characteristic nature of Shankhanjana, ensuring sensory consistency with classical descriptions.

2. PHYSICOCHEMICAL PARAMETERS

Alcohol Soluble Extractive

2 g of Shankhanjana sample was accurately weighed in a glass stoppered flask. 100 ml of distilled Alcohol (approximately 95%) was added and the flask was shaken occasionally for 6 hours, followed by standing for 18 hours. The mixture was filtered rapidly to avoid loss of solvent. 25 ml of the filtrate was pipetted into a pre-weighed 100 ml beaker, evaporated to dryness on a water bath, and kept in an air oven at 105°C for 6 hours. After cooling in a desiccator for 30 minutes, the residue was weighed. The experiment was repeated twice and the average percentage of alcohol soluble extractive was calculated.

Water Soluble Extractive

2 g of Shankhanjana sample was accurately weighed in a glass stoppered flask. 100 ml of distilled water was added and the flask was shaken occasionally for 6 hours, followed by standing for 18 hours. The mixture was rapidly filtered and 25 ml of the filtrate was pipetted into a pre-weighed 100 ml beaker. The filtrate was evaporated to dryness on a water bath and kept in an air oven at 105°C for 6 hours. After cooling in a desiccator, the residue was weighed. The experiment was repeated twice and the average percentage of water soluble extractive was calculated.

Determination of pH

Standard buffer solutions of pH 4, 7, and 9.2 were prepared by dissolving one tablet each in 100 ml of distilled water. 1 g of the Shankhanjana sample was taken and 10 ml of distilled water was added, stirred well, and filtered. The filtrate was used for the experiment. The pH meter was allowed to warm up for 30 minutes, calibrated sequentially using pH 4, 7, and 9.2 buffer solutions. The sample filtrate was then introduced and the pH reading was noted. The test was repeated four times and the average reading was taken as the result.

3. RESULTS OF PHYSICOCHEMICAL ANALYSIS

Table 1: Physico-chemical Parameters of Shankhanjana.

Parameters	Results (n=3, %w/w) Shankhanjana (Avg ± SEM)
Color	White
Odor	Pungent
Taste	Tingling and burning sensation in the tongue
Alcohol Soluble Extractive	1.61 ± 0.01
Water Soluble Extractive	6.54 ± 0.02
pH	7.5

The physicochemical parameters of Shankhanjana confirm its characteristic identity. The pH of 7.5 indicates a near-neutral to mildly alkaline nature, which is well within the acceptable range for ocular formulations and is consistent with the physiological pH of the conjunctival sac (approximately 7.4). The water soluble extractive value (6.54 ± 0.02% w/w) is considerably higher than the alcohol soluble extractive (1.61 ± 0.01% w/w), suggesting that the predominant active constituents are water-soluble in nature.

4. HPTLC PROFILE (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

High Performance Thin Layer Chromatography (HPTLC) was performed on the alcoholic extract of Shankhanjana to evaluate its chemical fingerprint. This technique enables qualitative identification, profiling,

and standardization of the formulation by visualizing phytoconstituents through chromatographic separation.

Sample Preparation and Methodology

- Sample: 1 g of Shankhanjana suspended in 10 ml of alcohol, kept for cold percolation for 24 hours, followed by filtration.
- Application volumes: 3 µl, 6 µl, and 9 µl applied on pre-coated silica gel F254 aluminum plates with a band width of 7 mm using Linomat 5 TLC applicator.
- Stationary phase: Silica gel 60 F254
- Mobile phase (Solvent system): Toluene: Ethyl acetate (9:1 v/v)
- Detection: Under Short UV (254 nm) and Long UV (366 nm); densitometric scanning at 254 nm and 366 nm; Rf values, color of spots, and densitometric scan were recorded.

Chromatographic Observations

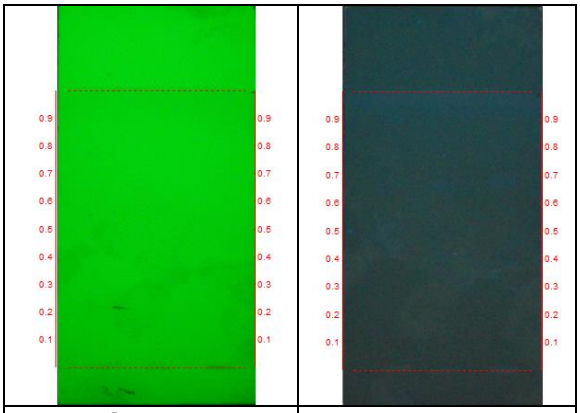


Figure 1: HPTLC photo documentation of ethanolic fraction of Shankhanjana.

Track 1 – Shankanjana – 3 µl
Track 2 – Shankanjana – 6 µl
Track 3 – Shankanjana – 9 µl
Solvent system – Toluene: Ethyl acetate (9:1)

Table 2: Rf Values of Shankhanjana at 254 nm and 366 nm.

At 254 nm	At 366 nm
Multiple peaks observed (Rf: 0.00 – 0.62)	Multiple peaks observed (Rf: 0.00 – 0.94)

A. Short UV (254 nm)

The HPTLC plate under short UV (254 nm) revealed the presence of multiple dark absorption bands across all three tracks. A total of 10 peaks were identified in the densitometric scan, with prominent peaks observed at Rf values of 0.47 (area 37.31%), 0.55 (area 24.77%), and 0.40 (area 12.81%). These compounds absorbing at 254 nm are typically UV-absorbing constituents such as aromatic compounds, phenolics, and alkaloids. The progressive increase in band intensity from Track 1 (3 µl) to Track 3 (9 µl) confirms concentration-dependent reproducibility.

B. Long UV (366 nm)

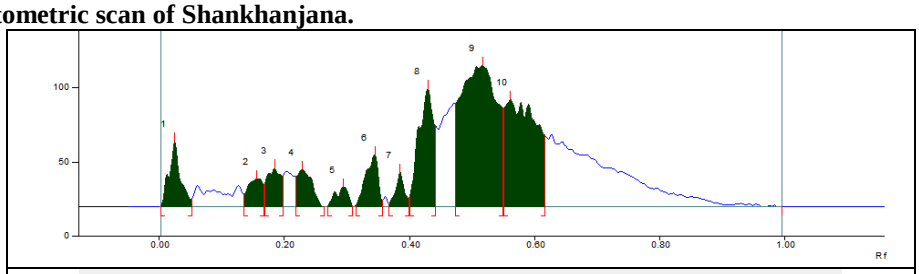
Under long UV (366 nm), the HPTLC plate revealed 14 distinct peaks in the densitometric scan. Prominent peaks were identified at Rf values of 0.71 (area 15.87%), 0.40 (area 15.03%), and 0.31 (area 11.41%). These fluorescent bands are characteristic of compounds such as flavonoids, terpenoids, coumarins, and volatile aromatic constituents. The appearance of these additional peaks at 366 nm highlights the richness and complexity of the phytochemical profile of Shankhanjana.

Significance of HPTLC Data

- The consistent visibility of multiple bands across all three tracks (3 µl to 9 µl) indicates a stable and reproducible chemical profile.
- The distinct Rf values observed are aligned with typical phytochemical constituents and mineral-based secondary metabolites, consistent with the nature of Anjana preparations.
- The intensity and clarity of bands under both UV detection wavelengths serve as a reliable chromatographic fingerprint for quality control and batch-to-batch standardization of Shankhanjana.
- The greater number of peaks at 366 nm compared to 254 nm further confirms the multicomponent chemical richness of this classical ophthalmic formulation.

5. DENSITOMETRIC SCANNING

Figure 2. Densitometric scan of Shankhanjana.

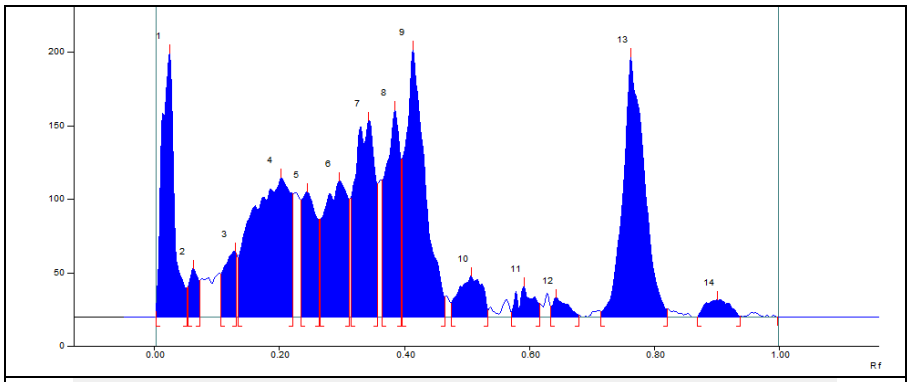


Track 3, ID: Shankanjana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.1 AU	0.02 Rf	44.3 AU	10.29 %	0.05 Rf	5.2 AU	571.4 AU	5.42 %
2	0.14 Rf	8.4 AU	0.16 Rf	18.9 AU	4.38 %	0.17 Rf	14.7 AU	327.0 AU	3.10 %
3	0.17 Rf	15.2 AU	0.19 Rf	26.0 AU	6.05 %	0.20 Rf	20.5 AU	414.2 AU	3.93 %
4	0.22 Rf	20.3 AU	0.23 Rf	24.7 AU	5.75 %	0.26 Rf	0.1 AU	427.6 AU	4.06 %
5	0.27 Rf	0.1 AU	0.30 Rf	13.0 AU	3.02 %	0.31 Rf	0.6 AU	188.5 AU	1.79 %
6	0.31 Rf	0.5 AU	0.35 Rf	34.5 AU	8.01 %	0.36 Rf	4.0 AU	493.2 AU	4.68 %
7	0.37 Rf	2.1 AU	0.39 Rf	22.9 AU	5.32 %	0.40 Rf	5.6 AU	224.6 AU	2.13 %
8	0.40 Rf	6.6 AU	0.43 Rf	79.3 AU	18.44 %	0.44 Rf	54.4 AU	1349.7 AU	12.81 %
9	0.47 Rf	69.4 AU	0.52 Rf	94.9 AU	22.07 %	0.55 Rf	66.2 AU	3931.6 AU	37.31 %
10	0.55 Rf	66.3 AU	0.56 Rf	71.7 AU	16.68 %	0.62 Rf	48.1 AU	2611.0 AU	24.77 %

Figure 2a – Densitogram at 254 nm (Short UV)

The densitometric scan at 254 nm revealed 10 peaks distributed across the Rf range of 0.00 to 0.62. The most prominent peaks were observed at Rf 0.47 (area% 37.31) and Rf 0.55 (area% 24.77), representing the major chemical constituents of the formulation. Additional peaks at Rf 0.40 (area% 12.81) and Rf 0.00 (area% 5.42) were also significant. Compounds absorbing at 254 nm are generally aromatic molecules including flavonoids, phenolic compounds, and alkaloids. This indicates the presence of chemically active principles with known antioxidant and anti-inflammatory properties.



Track 3, ID: Shankanjana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.9 AU	0.02 Rf	179.0 AU	14.50 %	0.05 Rf	19.7 AU	2550.6 AU	9.47 %
2	0.05 Rf	20.6 AU	0.06 Rf	33.0 AU	2.67 %	0.07 Rf	24.9 AU	357.7 AU	1.33 %
3	0.11 Rf	29.4 AU	0.13 Rf	44.8 AU	3.63 %	0.13 Rf	43.6 AU	609.9 AU	2.26 %
4	0.13 Rf	40.5 AU	0.20 Rf	94.6 AU	7.66 %	0.22 Rf	83.6 AU	4195.3 AU	15.57 %
5	0.24 Rf	79.5 AU	0.25 Rf	84.8 AU	6.87 %	0.26 Rf	66.4 AU	1461.3 AU	5.42 %
6	0.27 Rf	66.4 AU	0.30 Rf	92.3 AU	7.48 %	0.31 Rf	79.9 AU	2443.4 AU	9.07 %
7	0.31 Rf	80.5 AU	0.34 Rf	133.4 AU	10.81 %	0.36 Rf	90.4 AU	3074.8 AU	11.41 %
8	0.36 Rf	93.2 AU	0.39 Rf	140.4 AU	11.38 %	0.40 Rf	06.8 AU	2285.8 AU	8.48 %
9	0.40 Rf	107.4 AU	0.41 Rf	181.5 AU	14.70 %	0.47 Rf	13.9 AU	4049.6 AU	15.03 %
10	0.48 Rf	9.5 AU	0.51 Rf	28.0 AU	2.27 %	0.53 Rf	4.8 AU	732.5 AU	2.72 %
11	0.57 Rf	3.1 AU	0.59 Rf	21.1 AU	1.71 %	0.62 Rf	9.4 AU	352.7 AU	1.31 %
12	0.64 Rf	7.2 AU	0.64 Rf	13.2 AU	1.07 %	0.68 Rf	1.1 AU	225.7 AU	0.84 %
13	0.71 Rf	4.0 AU	0.76 Rf	176.6 AU	14.31 %	0.82 Rf	5.3 AU	4276.1 AU	15.87 %
14	0.87 Rf	0.0 AU	0.90 Rf	11.6 AU	0.94 %	0.94 Rf	0.5 AU	331.7 AU	1.23 %

Figure 2b – Densitogram at 366 nm (Long UV).

The densitometric scan at 366 nm revealed 14 distinct peaks, reflecting an even more complex chemical profile than observed at 254 nm. The most prominent peaks were noted at Rf 0.71 (area% 15.87), Rf 0.40 (area% 15.03), Rf 0.22 (area% 20.34), and Rf 0.31 (area% 11.41). Fluorescence at 366 nm is characteristic of terpenoids, flavonoids, essential oil components, and coumarins. The greater number of resolved peaks at this wavelength confirms the multicomponent and phytochemically rich nature of Shankhanjana. The dose-dependent consistency of peak patterns across the three application volumes (3, 6, and 9 µl) confirms excellent reproducibility of the formulation.

DISCUSSION

The present study focuses on the physicochemical standardization and HPTLC fingerprint profiling of Shankhanjana. In Ayurveda, the Anjana route of drug delivery is given prime importance in the management of Netra rogas because it allows direct contact of the drug with the ocular surface and facilitates rapid local action. Shankhanjana is traditionally used for conditions involving Kapha-dominant pathologies of the eye such as discharge, inflammation, microbial infection. Its characteristic Katu rasa, Sheeta virya, and Kapha-Vata shamaka properties make it a therapeutically significant preparation in classical ophthalmic practice.

The organoleptic evaluation confirmed that the sample was white in color, pungent in odor, and produced a tingling and burning sensation on the tongue, which is consistent with the classical description of Shankhanjana and indicative of its active mineral and herbal constituents. The white color and pungent characteristics are typical features of Anjana preparations containing mineral-based components.

The physicochemical analysis revealed that the water soluble extractive value (6.54 ± 0.02% w/w) was significantly higher than the alcohol soluble extractive (1.61 ± 0.01% w/w), indicating that the predominant active constituents of Shankhanjana are water-soluble in nature. The pH value of 7.5 reflects a near-neutral to mildly alkaline character, which is highly significant from the perspective of ocular safety and drug delivery. The physiological pH of the conjunctival sac ranges between 7.3 and 7.7, and a formulation with a pH of 7.5 closely approximates this range, thereby minimizing the risk of ocular irritation, discomfort, or damage to the corneal epithelium upon application. This near-physiological pH supports the suitability of Shankhanjana as an Anjana preparation for direct ocular use and is consistent with the classical emphasis on non-irritant and tolerable ophthalmic formulations.

Chemical fingerprinting by HPTLC demonstrated that Shankhanjana possesses a rich and complex phytochemical profile. At 254 nm, ten distinct peaks were resolved, with the most prominent compounds concentrated in the Rf range of 0.40–0.62. At 366 nm, fourteen peaks were identified, revealing additional fluorescent constituents such as terpenoids, coumarins, and aromatic volatile compounds not visible at the shorter wavelength. This increased resolution at 366 nm reflects the diverse chemical nature of the formulation, encompassing both UV-absorbing and fluorescent phytochemical classes. The detected compound classes, including phenolics, flavonoids, alkaloids, and terpenoids, are well known for their anti-inflammatory, antimicrobial, and antioxidant activities, which provide a pharmacological basis for the traditional use of Shankhanjana in ocular inflammatory and infectious conditions.

Densitometric scanning confirmed dose-dependent and consistent peak patterns across all three application volumes, establishing the reproducibility and homogeneity of the preparation. This reproducibility is critical for a classical ocular preparation, as uniformity in composition directly determines therapeutic reliability and safety. The developed HPTLC densitometric fingerprint can serve as a reliable analytical standard for future batch-to-batch quality verification of Shankhanjana.

Overall, the integrated analytical findings establish that Shankhanjana is a physicochemically characterized, analytically reproducible, and phytochemically rich classical ophthalmic preparation. The standardization parameters established in this study provide a scientific foundation for quality control, authentication, and wider clinical application of this important Ayurvedic Anjana formulation.

CONCLUSION

The present analytical study on Shankhanjana has successfully established its physicochemical and chromatographic profile. The organoleptic characters (white color, pungent odor, and tingling taste), near-neutral pH (7.5), and extractive values (alcohol soluble: 1.61 ± 0.01% w/w; water soluble: 6.54 ± 0.02% w/w) provide reliable baseline parameters for quality standardization. HPTLC analysis revealed a complex and reproducible fingerprint with multiple phytochemical constituents identified across both UV detection wavelengths (254 nm and 366 nm), including flavonoids, phenolics, alkaloids, terpenoids, and aromatic compounds. The densitometric scan offers a validated chromatographic fingerprint that can be used as a standard reference for the authentication and quality assurance of Shankhanjana in future preparations. These findings contribute to the scientific validation of classical Ayurvedic ophthalmic preparations and support the need for systematic standardization of Anjana formulations for contemporary clinical practice.

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