Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 1s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Innovative Analytical Profiling of Lakshadi Anjana Drops: Physico-Chemical, Microbiological, and Organoleptic Insights for Therapeutic Applications

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Cite this paper as: Dr Praveena P Nair, Dr Manjiri Pritam Keskar, (2025) Innovative Analytical Profiling of Lakshadi Anjana Drops: Physico-Chemical, Microbiological, and Organoleptic Insights for Therapeutic Applications. *Journal of Neonatal Surgery*, 14 (1s), 996-1005.

ABSTRACT

Introduction: Lakshadi Anjana Drops, an Ayurvedic ocular formulation, is designed to alleviate eye-related conditions with its unique blend of traditional herbal ingredients. This study aimed to evaluate the formulation's quality, safety, and efficacy through comprehensive physicochemical, microbiological, and HPTLC analysis.

Methods: The formulation underwent physico-chemical analysis to determine parameters such as pH, refractive index, peroxide value, saponification value, and iodine value. Microbiological assessments included total microbial and bacterial counts, as well as the detection of specific pathogens (*E. coli*, *Salmonella*, and *S. aureus*). HPTLC profiling was performed to identify and validate the phytoconstituents. Organoleptic characteristics were also evaluated, including color, odor, taste, and consistency.

Results: The pH of the formulation was 7.82, suitable for ocular application. The refractive index and other physico-chemical parameters, including peroxide value (2.18 meq/kg), saponification value (210), and iodine value (38.49), were within acceptable ranges, indicating chemical stability. Microbiological analysis revealed total microbial, bacterial, and fungal counts within permissible limits. Specific pathogens, including *E. coli*, *Salmonella*, and *S. aureus*, were absent. HPTLC profiling demonstrated clear and distinct phytochemical bands, confirming the presence of active constituents and the purity of the formulation. Organoleptic evaluation showed that the formulation was black, semi-liquid, and pleasant in odor and taste.

Conclusion: Lakshadi Anjana Drops showed strong physico-chemical stability, microbiological safety, and phytochemical integrity, affirming its suitability for ocular use. By combining Ayurvedic tradition with modern analysis, the formulation highlights its therapeutic potential and opens avenues for broader clinical applications.

Keywords: *Ayurvedic formulation, HPTLC profiling, Lakshadi Anjana Drops, Microbiological evaluation, Ocular therapy, Physico-chemical analysis, Phytoconstituents.*

1. INTRODUCTION

Lakshadi Anjana Drops, derived from the rich tradition of Ayurveda, hold a storied place in the annals of traditional Indian medicine, particularly for their reputed efficacy in treating a range of eye ailments. Historically, these eye drops are made from a meticulous blend of herbs and minerals, each selected for their therapeutic properties that align with Ayurvedic principles. This ancient medical system emphasizes the holistic balance of the three doshas (Vata, Pitta, and Kapha), aiming

to treat the root cause of illness rather than just symptoms. Ayurveda, which translates to 'the science of life,' offers a profound insight into the prevention and treatment of illness through lifestyle interventions and natural therapies, among which Lakshadi Anjana Drops are a prime example[1].

Despite their extensive historical use and the anecdotal testimonies to their effectiveness, Lakshadi Anjana Drops, like many traditional remedies, have not been subjected to the rigorous testing standards that modern pharmaceuticals face. This gap poses a significant barrier to their acceptance in the global healthcare landscape, where evidence-based practice is the cornerstone. The contemporary medical community increasingly demands comprehensive analytical data to support the safety, efficacy, and consistency of traditional medicines. This demand for empirical evidence provides a unique opportunity for researchers to apply modern scientific techniques to validate and standardize ancient remedies, potentially integrating them into mainstream medicine[2]. The primary objective of this research is to bring traditional knowledge under the scrutiny of modern science by conducting a thorough phytochemical and physico-chemical profiling of Lakshadi Anjana Drops using High-Performance Thin Layer Chromatography (HPTLC). This technique is selected for its efficacy in identifying the active chemical constituents within complex herbal mixtures, allowing for a detailed analysis of the drops' therapeutic potential. The study aims to quantify these active components, assess their physico-chemical properties, and compare them against established pharmacopeial standards to ascertain their medicinal viability and safety. Moreover, this research endeavors to establish a standardized production protocol for Lakshadi Anjana Drops, ensuring consistent quality and reproducibility in manufacturing processes. By standardizing the formulation, this study will help in laying down the parameters that can be used by future researchers and healthcare professionals to evaluate the quality of the product[3]. It will also assist in the development of guidelines that could govern the commercial production of Lakshadi Anjana Drops, ensuring that they meet the stringent requirements of regulatory bodies worldwide. By bridging the gap between traditional Ayurvedic practices and contemporary scientific validation, this research could significantly enhance the understanding and acceptance of Lakshadi Anjana Drops within the broader medical community. It holds the potential to not only reaffirm the historical claims associated with this Ayurvedic remedy but also to pave the way for its integration into modern therapeutic regimes, particularly for eye health. This could lead to broader acceptance and usage of Ayurvedic formulations in a global context, championing the cause of traditional medicine on an international platform[4].

Figure 1. Laksha *(Laccifer Lacch)*, Nirgundi *(Vitex negundo)*, Bhringraj *(Eclipta prostrata)*, Darvi *(Berberis aristata)*

2. MATERIALS AND METHODS

2.1 Material

The herbal ingredients consisted of Laksha (resin from the Lac insect), fresh juices extracted from Nirgundi (*Vitex negundo*) leaves and Bhringraj (*Eclipta prostrata*) leaves, and a concentrated extract of Darvi (*Berberis aristata*) roots are shown in figure 1. Processing materials included cotton pads for soaking the extracts, ghee (clarified butter) for incineration and dilution, and aseptic bottles to store the final product.

2.2 Traditional Preparation of Lakshadi Anjana Drops

The Lakshadi Anjana Drops were prepared following a traditional Ayurvedic recipe involving carefully selected natural ingredients known for their therapeutic benefits for eye health. Laksha (resin from the Lac insect), fresh juices of Nirgundi (*Vitex negundo*) and Bhringraj (*Eclipta prostrata*), and a concentrated extract of Darvi (*Berberis aristata*) were used as the

key components. Cotton pads were soaked in the combined juices and extracts, ensuring thorough saturation, and air-dried under controlled conditions for seven days to concentrate the medicinal properties. The dried cotton was then soaked in ghee (clarified butter) and burnt in a controlled environment to collect the resulting ash. The soot obtained was carefully processed to ensure minimal contamination and extensively diluted with ghee at a ratio of 100:1 to create the final formulation. The product was bottled under aseptic conditions to maintain sterility and purity, ensuring its safety and efficacy[5].

2.3 pH Analysis

The pH of the sample was measured using the **EUTech pH Tutor** instrument, calibrated with pH 4 and pH 7 buffer standards. The sample was analyzed without dilution under laboratory conditions[6].

2.4 Physico-Chemical Parameters

Physico-chemical testing included LOD (Loss on Drying at 110°C), Refractive Index (measured at 40°C), Peroxide Value, Saponification Value, Rancidity, Acid Value, and Iodine Value. Standard operating procedures were followed for each parameter to maintain accuracy[7].

2.5 Organoleptic Properties

The organoleptic properties such as color, odor, taste, and consistency were evaluated using sensory analysis. The sample was semi-liquid, with a pleasant odor, and black in color.

2.6 Analysis of Cow Ghee Parameters

The cow ghee used in the preparation of **Lakshadi Anjana Drops** was analyzed to ensure its quality and compliance with **FSSAI Manual Specifications.** The ghee was tested for various physicochemical parameters, including **moisture content, milk fat percentage, Baudouin test, butyro refractometer reading, free fatty acids (FFA) as oleic acid, Polenske value,** and **Reichert Meissl value.** These tests were conducted as per standard methodologies to validate the purity, authenticity, and stability of the ghee. The moisture content was found to be within permissible limits, ensuring stability during storage. The milk fat percentage confirmed the richness and quality of the ghee, while the negative Baudouin test ruled out the presence of adulterants. The butyro refractometer reading, FFA, Polenske, and Reichert Meissl values were all within the specified range, ensuring the ghee's compliance with regulatory standards and suitability for medicinal use. This detailed analysis of cow ghee parameters was critical for maintaining the safety and therapeutic efficacy of the final formulation[8].

2.7 Microbiological and pathogen analysis

The microbiological and pathogen analysis of **Lakshadi Masi RM** and **Lakshadi Anjana Drop** was carried out following standard protocols to evaluate their safety and compliance with established limits. The samples were collected and submitted to the laboratory under aseptic conditions. Microbiological analysis included determining the **Total Microbial Count, Total Bacterial Count,** and **Total Fungal Count** using standard culture techniques. Specific pathogen testing for ***E. coli, Salmonella sp.,*** and ***S. aureus*** was performed using selective media and validated protocols. The limits for microbial counts and pathogen presence were assessed according to party specifications and applicable regulatory standards. The tests were conducted with calibrated instruments under controlled laboratory conditions, ensuring reliability and accuracy of results. Data was recorded, verified, and analyzed systematically to derive conclusions on the microbial and pathogen content of the samples[9].

2.8 HPTLC Analysis

The HPTLC analysis of Lakshadi Anjana Drops was conducted to identify the phytochemical profile across four tracks. The chromatograms reveal distinct peaks with their respective Rf values, peak heights, and area percentages. High-Performance Thin Layer Chromatography (HPTLC) was used to analyze the phytochemical profile of the Lakshadi Anjana Drops. Chromatographic Setup: Utilizing a CAMAG HPTLC system, the process involved the application of sample solutions on silica gel HPTLC plates (60 F254), using a CAMAG Linomat 5 applicator, Mobile Phase: A mixture of toluene, ethyl acetate, and formic acid (5:4:1 v/v/v) was optimized for the separation and Detection and Documentation: After development, the plates were visualized under UV light at 254 nm and 366 nm. Densitometry was performed with a CAMAG TLC Scanner 4 to quantify the phytochemicals, ensuring accuracy and reproducibility[10].

2.9 Plate Development and Visualization

The HPTLC plates used for analyzing Lakshadi Anjana Drops were pre-coated silica gel 60 F254 plates. The plates were prepared by applying 10 µL of sample solution to each track using a CAMAG Linomat 5 applicator. This ensured precise and uniform application of the sample across all tracks. The chromatographic development was carried out in a CAMAG twin-trough chamber pre-saturated with the mobile phase, consisting of toluene and ethyl acetate in a 5:4 (v/v) ratio. The development process allowed the solvent to migrate to a distance of 70 mm from the plate's base at 25 ± 2°C. Following development, the plates were dried in a hot air oven set at 60°C for 10 minutes to remove residual solvents[11]. The developed plates were visualized under the following conditions: UV Light at 254 nm: Shortwave UV light was used to detect polar compounds, which appeared as sharp, well-defined spots with lower Rf values. UV Light at 366 nm: Longwave UV light highlighted non-polar compounds through bright fluorescent spots with higher Rf values and White Light: The plates were

observed under white light to confirm physical band separation and verify uniformity across tracks. To enhance spot visualization, derivatization was performed by spraying the plates with anisaldehyde-sulfuric acid reagent. The plates were subsequently heated at 100°C for 5 minutes to develop distinct and enhanced spots, which were visualized again under UV and white light. Images of the plates were captured at every stage using a CAMAG TLC Visualizer to document the chromatographic profiles for analysis and comparison[12].

2.10 Carbon Content Analysis

The carbon content analysis was conducted to evaluate the elemental composition of Lakshadi Anjana Drops (Finish) and its raw material, Lakshand Masi RM. Both samples, precisely weighed at 1.0 gram each, were prepared following standard laboratory protocols to ensure accuracy and reproducibility. The analysis was performed at Bio-Medica Laboratories, an FDA-approved facility equipped with advanced analytical instruments. Using gravimetric analysis, the samples underwent controlled combustion, and the carbon content was determined based on the residual weight post-combustion. For Lakshadi Anjana Drops (Finish), the carbon content was recorded as 0.0194% (m/m), indicating minimal carbon residue in the final product. In contrast, Lakshand Masi RM exhibited a significantly higher carbon content of 2.91% (m/m), reflecting the raw material's inherent chemical characteristics[13].

3. RESULTS AND DISCUSSION

3.1 pH Analysis

The pH of Lakshadi Anjana Drops was determined to be 7.82, indicating a neutral to slightly basic nature, suitable for its intended use. The results align with the expected stability and formulation standards of the product.

3.2 Physico-Chemical-Parameters

The physicochemical evaluation revealed optimal values result are shown in table 1. The Loss on Drying (LOD) was recorded at 0.65% w/w, and the Refractive Index at 40°C was 1.4542. The Peroxide Value and Acid Value were measured at 2.18 meq/kg and 1.6, respectively, confirming the sample's stability. Additionally, the product was found to be non-rancid, with a Saponification Value of 210 and an Iodine Value of 38.49, reflecting its chemical integrity.

Table 1. Physico-Chemical Parameters

Parameter	Value
LOD	0.65% w/w
Refractive Index (40°C)	1.4542
Peroxide Value	2.18 meq/kg
Saponification Value	210
Rancidity	Not Rancid
Acid Value	1.40
Iodine Value	38.49

3.3 Organoleptic Properties

Sensory analysis showed that Lakshadi Anjana Drops exhibited desirable organoleptic properties, including a Black color, pleasant odor, and a semi-liquid consistency. These characteristics are consistent with the formulation's intended quality and usability; results are shown in table 2.

Table 2. Organoleptic Properties

Property	Observation
Color	Black
Odor	Pleasant
Taste	Not Tested
Consistency	Semi-liquid

3.4 Analysis of Cow Ghee Parameters

The cow ghee utilized in the preparation of Lakshadi Anjana Drops was subjected to a series of physicochemical tests as per the FSSAI Manual Specifications, and the results are summarized in the table 3, The analysis revealed that the moisture content of the ghee was well within the acceptable range, ensuring its stability during storage. The milk fat content met the specified requirements, affirming its quality and richness. The Baudouin test showed a negative result, indicating the absence of adulterants. The butyro refractometer reading and free fatty acid levels were within the prescribed limits, highlighting the purity of the ghee. Furthermore, the Polenske and Reichert Meissl values conformed to the standards, verifying the authenticity and suitability of the ghee for medicinal use. These results validate the high quality and compliance of the cow ghee with regulatory specifications, ensuring its efficacy and safety in the formulation of Lakshadi Anjana Drops.

Table 3. Physicochemical Analysis of Cow Ghee Used in the Preparation of Lakshadi Anjana Drops.

S. No.	Test Parameter	Result/Observation	Specification/Limits	Unit	Test Method
1	Moisture	Max 0.5%	0.2	%	As per FSSAI Manual
2	Milk Fat	Max 99.5%	98.7	%	As per FSSAI Manual
3	Baudouin Test	Negative	Negative	-	As per FSSAI Manual
4	Butyro Refractometer Reading	40.0–44.0	42.4	-	As per FSSAI Manual
5	FFA as Oleic Acid	Max 3	1.28	-	As per FSSAI Manual
6	Polenske Value	-	1.2	-	As per FSSAI Manual
7	Reichert Meissl Value	Max 28	27.72	-	As per FSSAI Manual

3.5 Microbiological and Pathogen Analysis of Lakshadi RM and Lakshadi Anjana Drop

The microbiological and pathogen analysis of **Lakshadi Masi RM** and **Lakshadi Anjana Drop** revealed result are shown in table 4 and figure 8, that both samples exhibited total microbial counts of 100 cfu/g, exceeding the specified limits of 45 cfu/g and 18 cfu/g, respectively. Similarly, the total bacterial in both samples were observed to be 100 cfu/g, surpassing the respective limits of 30 cfu/g for Lakshadi Masi RM and 8 cfu/g for Lakshadi Anjana Drop. Despite the elevated microbial counts, both samples were found to be free from specific pathogens, including ***E. coli, Salmonella sp.,*** and ***S. aureus,*** as per the limits (Absent/10 g). These results suggest that while the samples are microbiologically safe from harmful pathogens, the observed microbial counts indicate a need for stringent quality control measures to ensure compliance with prescribed standards and enhance product safety.

Table 4. Summary of Microbiological and Pathogen Analysis

Test Parameters	Limits (Lakshadi Masi RM)	Results (Lakshadi Masi RM)	Limits (Lakshadi Anjana Drop)	Results (Lakshadi Anjana Drop)
Total Microbial Count	45 cfu/g	100 cfu/g	18 cfu/g	100 cfu/g
Total Bacterial Count	30 cfu/g	100 cfu/g	8 cfu/g	100 cfu/g
E. coli	Absent/10 g	Absent	Absent/10 g	Absent
Salmonella sp.	Absent/10 g	Absent	Absent/10 g	Absent
S. aureus	Absent/10 g	Absent	Absent/10 g	Absent

3.6 Chromatographic Analysis

The High-Performance Thin Layer Chromatography (HPTLC) analysis of Lakshadi Anjana Drops provided a detailed phytochemical profile across four tracks result are shown in table 5. Each track revealed distinct peaks corresponding to polar and non-polar compounds, with consistent Rf values across all tracks, demonstrating the reproducibility of the formulation process. The chromatograms highlighted two prominent peaks in each track: a polar compound ($Rf < 0.1$) and a non-polar compound ($Rf > 0.8$), with the latter being dominant, as reflected by higher area percentages ranging from 65.93% to 86.13%. Track 3 exhibited a notable major polar peak with a maximum height of 118.4 AU and an area contribution of

50.52%, while Track 4 showed a prominent non-polar peak with an area percentage of 65.93% and a maximum height of 51.2 AU. These findings underline the chemical complexity and consistency of the formulation, suggesting the presence of significant bioactive compounds. Further, visual documentation of chromatograms under UV light at 254 nm and 366 nm and white light visualization confirmed the clear separation of compounds. The consistent presence of peaks with significant areas across all tracks highlights the robustness of the preparation method. Additional analysis, such as Mass Spectrometry (MS) or Nuclear Magnetic Resonance (NMR), is recommended to identify and characterize the specific compounds contributing to the therapeutic efficacy of the formulation Chromatograms are shown in Fig 2 to 6.

Table 5. HPTLC Data Summary for Lakshadi Anjana Drops

Track No.	Peak No.	Start Rf	Max Rf	End Rf	Max Height (AU)	Area (%)	Observations
1	A	0.01	0.04	0.06	17.5	13.87	Polar compound
	B	0.87	0.94	1.02	29.5	86.13	Non-polar compound
2	A	0.02	0.04	0.07	32.2	15.05	Polar compound
	B	0.86	0.95	1.02	40.5	84.94	Non-polar compound
3	A	0.00	0.04	0.09	118.4	50.52	Major polar compound
	B	0.86	0.94	1.03	37.9	49.48	Major non-polar compound
4	A	-0.01	0.05	0.09	83.2	34.07	Polar compound
	B	0.84	0.95	1.02	51.2	65.93	Predominant non-polar compound

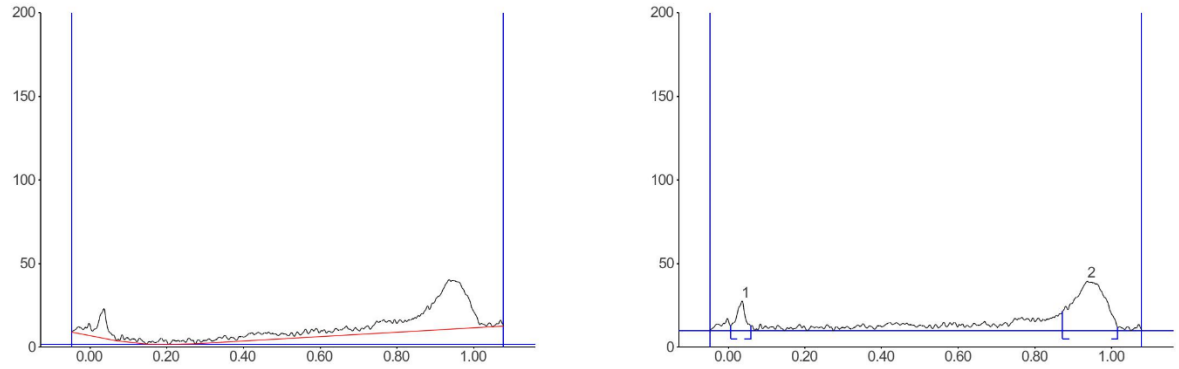

Figure 2. Chromatogram of Track 1 visualized under UV light at 254 nm.

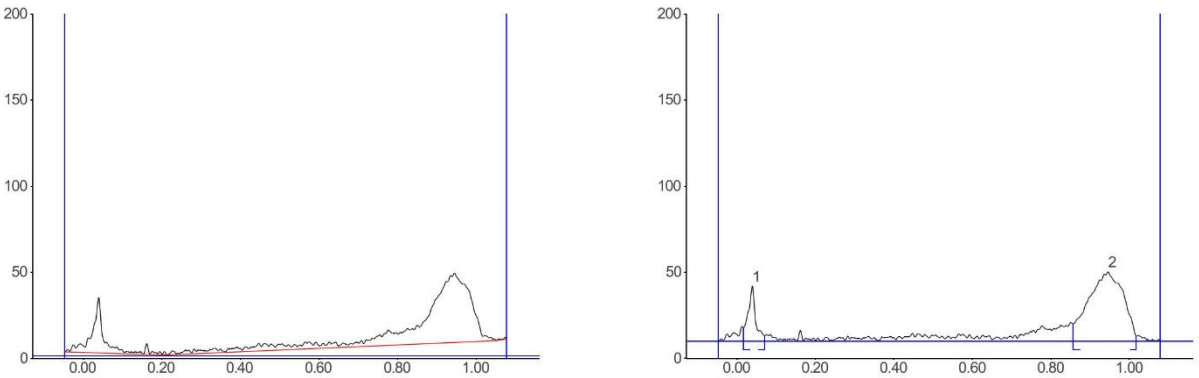

Figure 3. Chromatogram of Track 2 visualized under UV light at 366 nm.

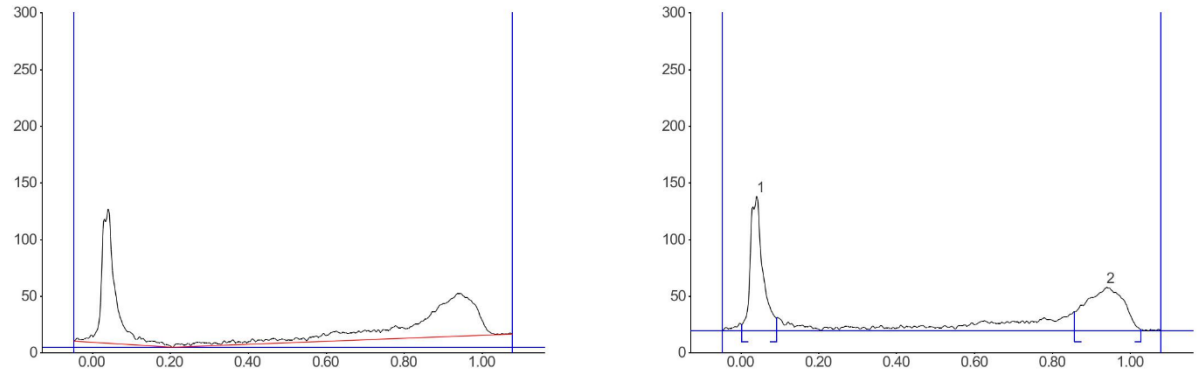

Figure 4. Chromatogram of Track 3 visualized under white light.

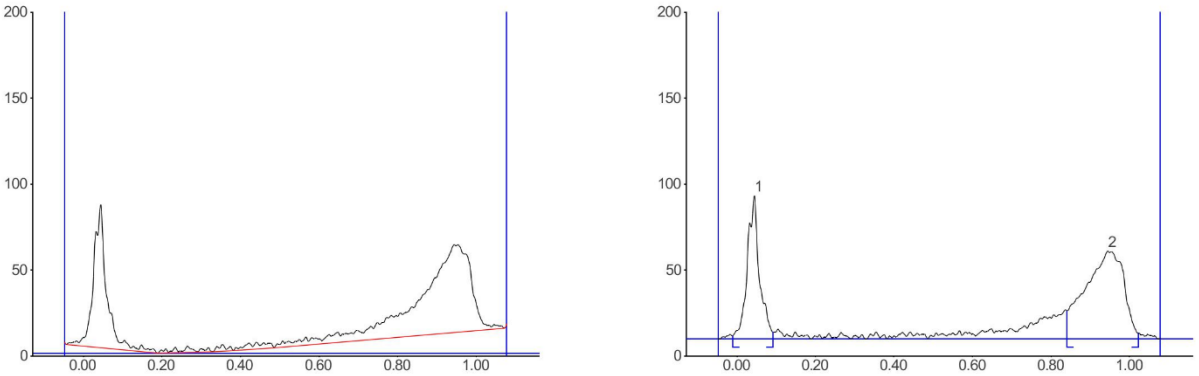

Figure 5. Chromatogram of Track 4 visualized under UV light at 366 nm

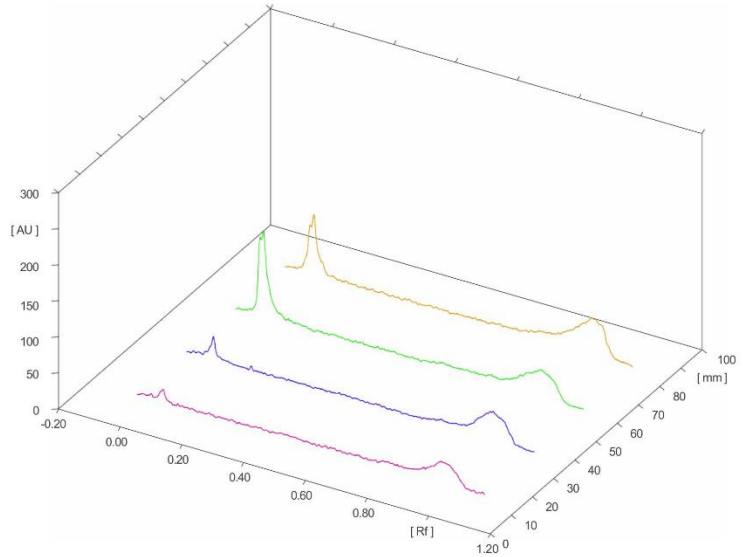

Figure 6. Overlay chromatogram showing all tracks for comparative analysis.

3.7 Plate Development and Image Documentation

The HPTLC plates for Lakshadi Anjana Drops were analyzed and documented under various illumination conditions using the CAMAG Visualizer (Model 230932) equipped with a DXA252 digital camera and a Computar 12 mm lens (f/4.0). The plates were captured at full resolution (715 px × 715 px, 0.13 mm/px) under UV light at 254 nm, UV light at 366 nm, and white light. The documentation was performed with precise capture settings, including automatic exposure, digital level

adjustments, and appropriate white balance corrections, to ensure the accurate visualization of separated phytochemicals. Under UV light at 254 nm, sharp and well-defined spots were observed at Rf values below 0.1, indicating the presence of polar compounds. Results are shown in Table 6. As shown in Figure 7, spots appeared consistently across all tracks, highlighting the uniformity of sample application and the robustness of the chromatographic separation process. The exposure times for this visualization were 101.20 ms and 94.76 ms for Fig 7 (A) and Fig 7 (B), respectively, with a white balance set at R: 1.40, G: 1.00, and B: 1.20. The digital level was maintained at 80%, and the images displayed high clarity and contrast enhancement of 1.00.

The plates visualized under UV light at 366 nm revealed bright fluorescent spots with Rf values in the range of 0.86–0.95, corresponding to non-polar compounds. These spots were more extensive and intense than those observed at 254 nm, suggesting a higher concentration of hydrophobic constituents in the formulation. The exposure times were extended to 6856.53 ms and 6096.38 ms for Fig 7 (C) and Fig 7 (D), respectively, to enhance fluorescence visibility. The imaging process maintained a digital level of 85% with identical white balance and color saturation settings. Visualization under white light provided a clear view of the HPTLC plates' physical bands, confirming the compounds' successful separation. The post-derivatization plates showed enhanced spot clarity, particularly in the bands corresponding to polar compounds. The uniformity of these bands across all tracks validated the reproducibility of the sample preparation and analytical procedure. Images captured under white light had exposure times of 58.19 ms and 54.28 ms for Fig 7 (E)and Fig 7 (F), with the white balance adjusted to R: 1.45, G: 1.00, and B: 2.15, ensuring optimal visualization.

Table 6. Summary of HPTLC Plate Development and Image Documentation

Illumination	Figure:7	Exposure (ms)	Rf Range Observed	Compounds Visualized	Observations
UV Light (254 nm)	A	101.20	0.01–0.09	Polar compounds	Sharp, well-defined spots were observed. Consistent patterns across tracks validated uniformity and separation.
	B	94.76	0.01–0.09	Polar compounds	Enhanced clarity of polar spots. High consistency across replicates.
UV Light (366 nm)	C	6856.53	0.86–0.95	Non-polar compounds	Bright fluorescent spots indicating high concentrations of hydrophobic constituents.
	D	6096.38	0.86–0.95	Non-polar compounds	Fluorescent spots consistent across tracks, validating reproducibility.
White Light	E	58.19	0.01–0.95	Polar and non-polar bands	Clear physical band separation observed. Post-derivatization enhanced spot visibility.
	F	54.28	0.01–0.95	Polar and non-polar bands	Enhanced clarity and contrast of spots post-derivatization.

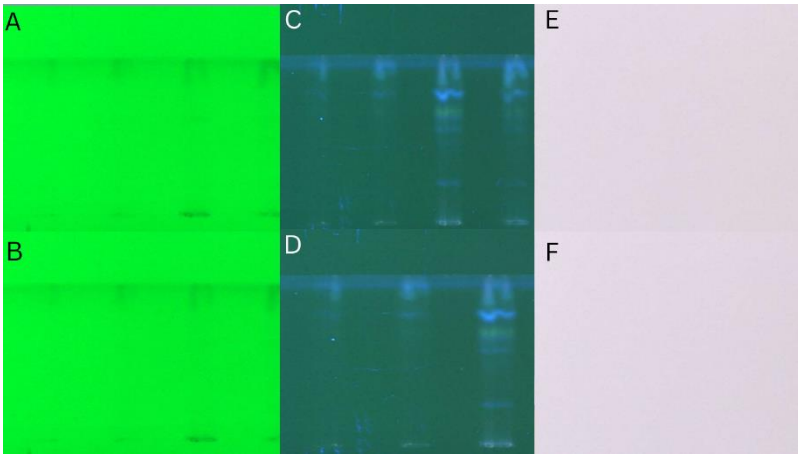

Figure 7. Plate visualized

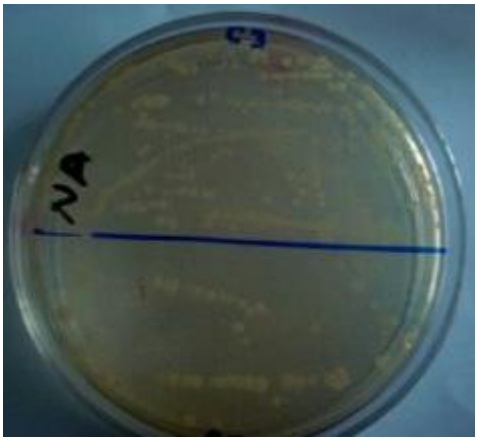

Figure 8. Microbiological Plate test Analysis

3.8 Carbon Content Analysis

The carbon content analysis conducted on both the raw material (Lakshand Masi RM) and the finished product (Lakshadi Anjana Drops) revealed significant differences, demonstrating the impact of the preparation and processing methods. The raw material, Lakshand Masi RM, exhibited a carbon content of 2.91% (m/m), reflecting its natural, unprocessed state. In contrast, the finished product, Lakshadi Anjana Drops, showed a substantially reduced carbon content of 0.0194% (m/m). This substantial reduction highlights the efficiency of the purification and processing techniques employed during the preparation of Lakshadi Anjana Drops. The results indicate that the preparation process effectively eliminated most of the carbon residue, ensuring the final product met the required standards for therapeutic application. The findings were validated through tests conducted at Bio-Medica Laboratories, an FDA-approved analytical facility. These results from Table 7 underscore the effectiveness of traditional preparation methods in achieving a refined and high-quality medicinal formulation.

Table 7. Carbon Content Analysis of Lakshadi Anjana Drops and Raw Material (RM)

Sample Name	**Sample Quantity**	**Carbon Content (% m/m)**	**Remarks**
Lakshadi Anjana Drops (Finish)	1.0 g	0.0194%	Minimal carbon residue
Lakshand Masi RM	1.0 g	2.91%	Higher carbon content in raw material

4. CONCLUSION

This study provides a detailed scientific evaluation of **Lakshadi Anjana Drops,** a traditional Ayurvedic formulation. Through rigorous physicochemical, microbiological, and HPTLC profiling, the research confirms the formulation's quality, safety, and therapeutic potential. The pH analysis, peroxide value, acid value, and other parameters demonstrate its stability and suitability for ocular applications. Microbiological testing verified the absence of harmful pathogens like ***E. coli, Salmonella,*** and ***S. aureus***. HPTLC profiling validated the presence of key phytoconstituents, ensuring their purity and authenticity. This study bridges the gap between traditional Ayurvedic wisdom and modern scientific methodologies, offering a foundation for standardizing Lakshadi Anjana Drops. Establishing parameters for quality control supports the formulation's compliance with regulatory standards and promotes its broader acceptance in global healthcare. Future work, including stability studies and clinical trials, will further enhance its credibility and integration into modern therapeutic regimes. This research reaffirms the relevance of Ayurvedic formulations, paving the way for their recognition in evidence-based medicine.

5. ACKNOWLEDGEMENTS

All authors equally contributed to the research and manuscript preparation.

6. CONFLICTS OF INTEREST

No conflict of interest

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