

**Formulation, Evaluation and Standardization of Phytopharmaceutical Dosage Form
with Anti-Infective Activity**

PhD Synopsis

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Title: Formulation, Evaluation and Standardization of Phytopharmaceutical Dosage Form with Anti-Infective Activity**Abstract**

The current global health landscape is increasingly defined by the escalation of antimicrobial resistance, necessitating the transition from empirical traditional medicine to evidence-based, regulated phytopharmaceuticals. This research investigates the therapeutic potential of *Kigelia africana* fruit extract, addressing the critical gap between ethnobotanical utility and modern pharmaceutical standardization.

The study's methodological rigor is anchored in the development of validated High-Performance Thin-Layer Chromatography (HPTLC) methods for the simultaneous and precise quantification of four key markers: verbascoside, caffeic acid, ferulic acid, and coumaric acid. To optimize extraction efficiency, a 3² full factorial Design of Experiments (DOE) was implemented to analyze the synergistic effects of solvent composition and duration. The optimized extract, was rigorously screened via Headspace-Gas Chromatography (HS-GC) to ensure residual ethanol remained well below the minimum inhibitory concentration (MIC) for relevant pathogens. This ensures that the observed antimicrobial efficacy is intrinsic to the phytochemical profile rather than the solvent. This systematic approach transforms extraction from a simple preparation step into a strategic determinant of therapeutic quality.

The subsequent development of a topical gel utilized a Quality by Design (QbD) framework, systematically linking material attributes to Critical Quality Attributes (CQAs). The resulting dosage form demonstrated significant *in-vitro* activity against *Staphylococcus aureus* and *Escherichia coli*. Accelerated stability studies conducted under ICH Q1A(R2) guidelines confirmed the formulation's physical and chemical integrity, supporting a projected shelf life of three years.

This thesis offers a sophisticated scientific framework for the translational development of plant-derived anti-infective. By integrating advanced analytical validation with engineering-driven optimization, it provides a scalable, standardized pathway for botanical medicines to address the therapeutic void left by failing synthetic alternatives.

Brief description of state of art of the research topic

Phytopharmaceuticals, are plant-derived therapeutic agents that must meet the same rigorous standards of quality, safety, and efficacy as conventional new drugs. Their development bridges traditional knowledge with modern regulatory frameworks, offering opportunities to generate evidence-based botanical medicines.

The modern day medicine available for fungal and bacterial skin disorders have and are becoming resistant to anti-infective (anti-fungal and antibacterial) drugs being prescribed. Usage of traditional medicines have more prone pharmacological actions with lesser side effects, adverse reactions and no toxicological implications. Combining traditional medicines with the modern synthetic chemicals as a new drug, establishing their safety, efficacy and activity carve out some new alternative medicine pathways. They have sufficient reliability ensuring patient safety and minimum side effects, adverse reactions and below par toxicological implications. Hence, regulatory agencies around the globe have been publishing guidelines for industrial standards for botanical origin. This makes it crystal clear that an alternative medicine pathway in underway wherein traditional medicines being used in specific regions can be linked to scientific knowledge and be commercialized.

Analytical methods have been developed and validated for the estimation and quantification of markers present in *Kigelia africana* fruit extracts. Our findings during optimization of the extraction process reveal a nuanced relationship between the investigated factors and the concentration of each marker. *In vitro* activity against relevant fungal and bacterial strains has been established, demonstrating the potential of the formulation as an effective topical anti-infective agent.

The proposed project stands out because it directly addresses two intersecting gaps: the global rise of fungal and bacterial resistance, and the underutilization of phytopharmaceuticals within regulated drug frameworks. While antifungal resistance has been identified as a growing global health threat, few research initiatives explore topical phytopharmaceuticals as credible therapeutic options. Most plant-based formulations remain confined to traditional or alternative medicine, without systematic validation against modern regulatory standards. This project moves beyond ethnobotanical claims by subjecting a topical gel to rigorous physical, chemical, and

pharmacological evaluation aligned with the Drugs and Cosmetics Act. That combination of traditional knowledge, pharmaceutical development, and regulatory compliance gives the study a distinctive edge.

The novelty also lies in its translational potential. Preliminary *in-vitro* findings already suggest promising antimicrobial activity, but the project does not stop at laboratory validation. By designing a dosage form with regulatory approval in view, it creates a pathway from bench to bedside, something rare in phytomedicine research, which often stalls at the proof-of-concept stage. The project's focus on a standardized, safe, and effective gel, positions it as more than an academic exercise: it offers a realistic, scalable product that could eventually fill a therapeutic gap in managing resistant fungal and bacterial infections.

Definition of the problem

Bacterial and fungal diseases affect hundreds of millions worldwide and contribute substantially to illness and premature death. Yet they remain under-recognized compared to bacterial or viral infections. With resistance to current antifungals increasing and only a handful of effective drugs available, there is an urgent demand for new approaches grounded in both safety and accessibility.

Healthcare practitioners have a tremendous dilemma in choosing anti-fungal agents as the prevalence of fungus infections rises alarmingly. Resistance happens when a medicine used to treat a fungal infection is no longer effective. Some fungal species are inherently resistant to being treated with specific antifungal medications.

Traditional medicinal plants represent an under-explored reservoir of therapeutic compounds. Many have a long history of cultural use that suggests acceptable safety, but their potential as standardized phytopharmaceuticals has not been fully evaluated. Bioactive plant-derived molecules are known to disrupt microbial growth and pathogenic mechanisms, offering a rational basis for targeted anti-infective action.

Objectives and scope of work

Aim: To develop formulation, standardize, & evaluate phytopharmaceutical dosage form with anti-infective activity.

Objectives:

1. To develop a suitable analytical method for estimation of selected markers.
2. To perform optimize the extraction process using design of experiments.
3. To evaluate the optimized extract for its chemical constituents and *in vitro* activity.
4. To develop topical dosage form containing optimized extract of *K. africana*.
5. To evaluate the optimized formulation for its physicochemical properties and *in vitro* activity.

Scope of work

The developed topical phytopharmaceutical gel can be used for topical skin infections. This project focuses on the formulation, evaluation and standardization of a phytopharmaceutical dosage form formulated from *K. africana* with documented antimicrobial properties. The formulation is a gel intended for external application, designed to provide targeted delivery at the site of infection while minimizing systemic exposure and associated adverse effects.

Original contribution by the thesis

The entire work in this synopsis and thesis is original. Literature review showed the better use of ethnopharmacological traditional use of an African native plant into modern medicine for fighting against anti-microbial resistance for topical skin infections.

More than one billion people worldwide suffer from fungus-related illnesses. Fungal infections affect over one billion people annually worldwide, with an estimated 3.8 million deaths reported in 2023–24. The developed topical gel is advantageous as an adjuvant therapy at the moment.

The analytical methods developed and validated benefit in quality control of *K. africana* and also in fingerprinting. the optimization extraction process for herbal materials is not merely a preparative technique but critical determinant of product quality, efficacy, and

sustainability. An optimized extraction protocol tailored to the specific phytochemical profile of the plant material is essential for producing high-value herbal formulations.

Methodology of research, results/comparisons

1) Analytical methods

Standardisation is a cornerstone of phytopharmaceutical development, ensuring reproducibility and quality. For *Kigelia africana*, four marker compounds are particularly significant: verbascoside (VBS), caffeic acid (CA), ferulic acid (FA), and coumaric acid (COA). Verbascoside, a phenylethanoid glycoside, exhibits potent antioxidant, anti-inflammatory, and antimicrobial activities. Its well-defined chemical structure and ease of detection via HPTLC methods make it an ideal marker. Caffeic acid, a hydroxycinnamic acid, has recognised antimicrobial and antioxidant effects, and serves as a useful indicator of phenolic content. Ferulic acid and coumaric acid, structurally related cinnamic acids, contribute to free radical scavenging, enzyme inhibition, and microbial suppression.

Analytical methods have been developed and validated for these markers, enabling quantification and quality control of *Kigelia* extracts. Simultaneous separation of all four target biomarkers proved unsuccessful. This was primarily due to the high polarity of VBS, in contrast to the structural and chemical similarity among the remaining three markers. Preliminary chromatographic trials did not reveal a single set of conditions suitable for concurrent resolution of all four compounds. Two distinct chromatographic methods: Method 1 for the estimation of VBS, and Method 2 for the simultaneous estimation of CA, COA, and FA.

Method-1 provided resolved and symmetric bands for VBS (R_F 0.15).

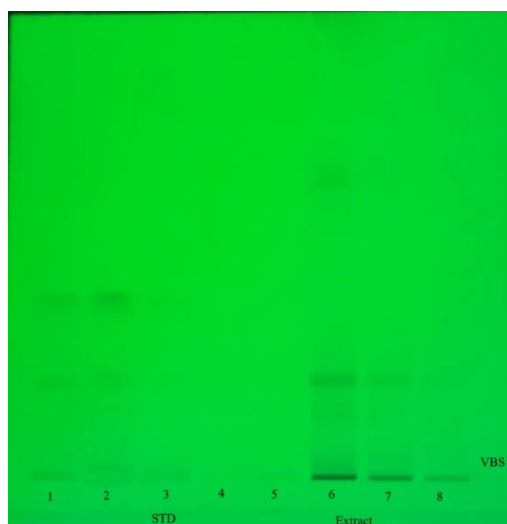


Figure 1 TLC photograph of VBS

The densitogram of standard solution showing the separated peaks using chromatographic conditions (Figure 2). Respective R_F value of VBS was found to be similar in the densitograms obtained from standard and sample solutions.

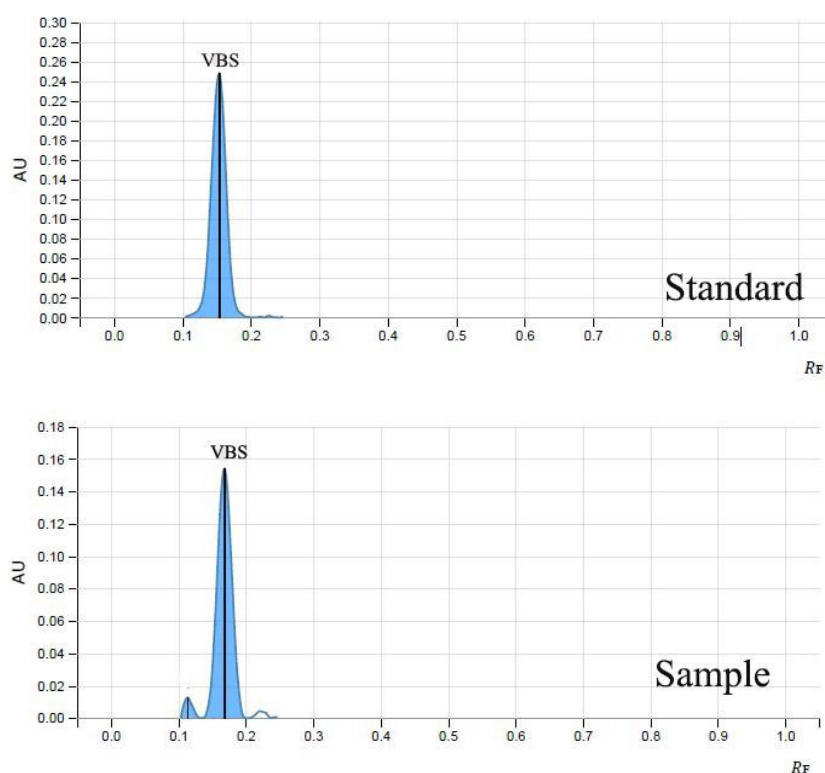


Figure 2 Densitogram of standard and sample VBS solution by method-1

The validation parameters for Method -1, i.e. precision was $< 2\%$ RSD and linear ($r^2 > 0.98$) and specific.

Method -2 was developed and validated for estimation of CA, COA and FA.

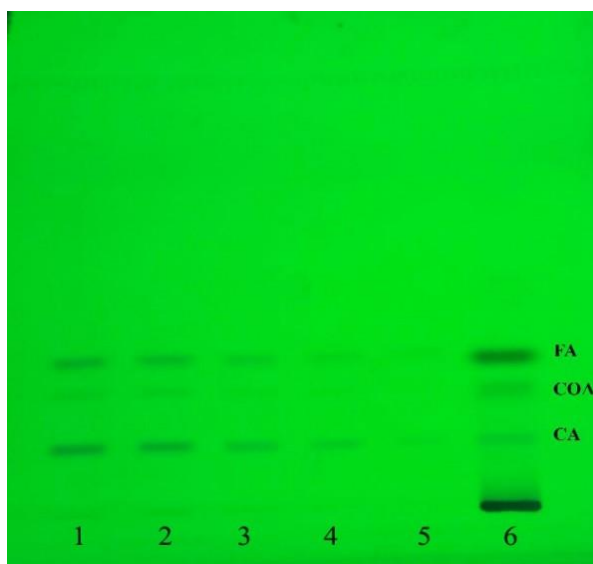


Figure 3 TLC Photograph of CA, COA and FA

Respective R_F values of CA, COA and FA were found to be similar in the densitograms obtained from standard and sample solutions (Figure 4).

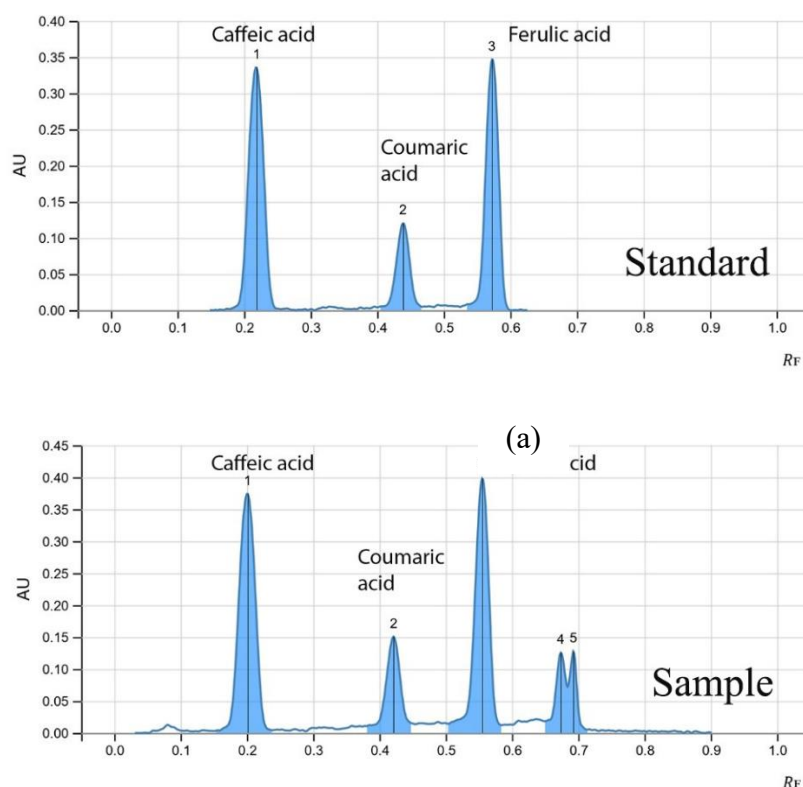


Figure 4 Densitogram of standard and sample solutions by method-2

The developed and validated method -2 for estimation of CA, COA and FA had RSD < 2 % for the validation parameters.

2) Optimization of extraction process

Extracting bioactive compounds from plant materials involves navigating a complex interplay of factors that significantly influence the yield and quality of the final extract. The extraction process is a fundamental step in the recovery and purification of bioactive compounds from herbal materials and has a direct impact on the pharmacological efficacy, stability, and formulation of herbal products. Phytochemicals present in herbs are part of complex matrices and are often sensitive to heat, light, and oxidation. Hence, extraction procedures must be designed to maximize yield while preserving chemical integrity and biological activity.

The complexity of herbal matrices necessitates a nuanced approach to solvent selection and process conditions. Optimizing the solvent selection is essential to achieving high extraction yields while preserving the integrity and potency of the extracted bioactive compounds. The varying composition of solvents, such as ethanol, water mixtures, affects the selectivity and yield of bioactive compounds with antimicrobial and antioxidant properties. Sufficient extraction time is essential to enable the complete dissolution and diffusion of the target compounds from the plant matrix into the solvent medium.

DOE empowers the development of models which provide quantitative relationships between input parameters and desired outputs, facilitating precise process control and optimization. In the context of herbal extractions, DOE plays a crucial role in fine-tuning key parameters like solvent composition, temperature, extraction time, and solid-to-liquid ratio to enhance yield and bioactivity.

To optimize the extraction process, a 3^2 full factorial design with four responses was employed through Design-Expert® software which generated experimental runs and analyses was done in triplicates enabling systematic evaluation of the main and interactive effects of two critical process parameters: Factor A- solvent composition (% ethanol, three levels: 50%, 75% and 100%) and Factor B - extraction time (three levels: 18 h, 36 h and 54h). These experimental runs were performed using soxhlet extractor. Common design space for all responses was generated graphically wherein each response criteria were set at certain limits depending upon the RSM graphs obtained and as to get a maximum concentration response for each and the combined overlay plot was generated which was validated. The robust validation of the predictive models, evidenced by correlation coefficients exceeding 0.99 between predicted and actual values, attests to the high accuracy and reliability of this experimental design approach.

Factor Coding: Actual

Overlay PlotVBS
CA
FA
COA

● Design Points

X1 = A

X2 = B

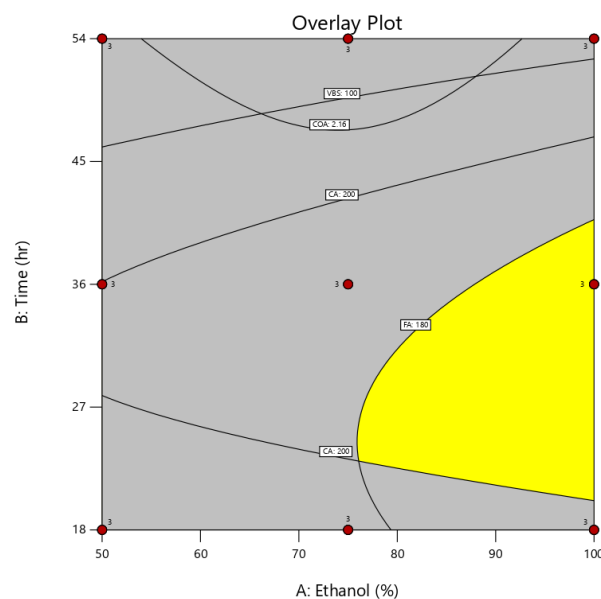


Figure 5 Overlay plot of the design

The application of DOE minimizes the experimental burden while maximizing the generation of actionable insights, thereby enabling the precise tuning of extraction processes to augment yield, enhance bioactivity, and improve overall operational efficiency. The validated optimized extract (ethanol 90 % and time 30 hrs) was to be taken as a raw material for topical formulation, hence it was evaluated on certain parameters

3) Evaluation of optimized extract

The optimized extract was evaluated on three parameters which form as a crucial basis for topical formulation as a raw material. The three parameters; Ethanolic content of the extract, *In-vitro* activity of the extract and Assay of the extract.

3.1) Ethanolic content of optimized extract

Headspace-Gas Chromatography (HS–GC) is predominantly used to determine the volatile contents present in a sample. Ethanol being volatile in nature, HS–GC was, by default, used to estimate it in dried optimized extract. The ethanolic content of the optimized extract analyzed by HS–GC was found to be under the minimum inhibitory concentration (MIC) limits reported in the literature review. Ethanol's MIC against *E. coli* and *S. aureus* is reported to be 8.75 %. The optimized extract's ethanolic content was calculated and found to be 6.8 %.

Table 1 Ethanolic content results

Sample	RT (min)	Area	Ethanol (%)
Ethanol (Standard)	4.386	2617.96	100
Optimized extract	4.390	34877.59	6.8

3.2) *In-vitro* activity of the optimized extract

In-vitro activity results of the optimized extract and ethanol (6.8%) as a control obtained against *E. coli* and *S. aureus* showed that the optimized extract was effective against both the species. Gentamicin as a standard, blank (sterile water) as a control were used in the study. The results display the effectiveness of the optimized extract against both the species.

3.3) Assay of the optimized extract

Method - 1 was used to dried optimized extract to determine the VBS contents. The dried optimized extract was found to contain 130.5 µcg/mL (0.12 % w/w) of VBS. Method-2 was applied to dried optimized extract to determine the CA, COA and FA contents. The dried optimized extract was found to contain 236.8 µg/mL (0.22 % w/w) of CA, 21.4 µg/mL (0.19 % w/w) of COA and 197.7 µg/mL (0.182 % w/w) of FA.

Evaluation of the optimized extract bridges the gap between essential characteristics required for extract as a raw material and the topical dosage form. The optimized extract point can now be taken as a raw material for development, evaluation and standardization of the phytopharmaceutical topical dosage form.

4) Formulation

The development of topical gels involves multiple product and process-related variables, each of which can significantly affect the safety, efficacy, and overall quality of the final formulation. Quality by Design (QbD) represents a systematic, science-based, and risk-oriented approach to pharmaceutical development. Its central principle is that product quality should not be tested into the final product but must instead be built into the design from the earliest stages of development.

In the present study, the QbD approach was employed for the development of phytopharmaceutical gel. By systematically identifying the QTPP, CQAs, CMAs, and CPPs, and applying risk assessment, the study sought to establish a well-optimized formulation with consistent quality attributes. This methodology ensures that the final product achieves the desired therapeutic outcomes while maintaining regulatory compliance.

5) Stability studies

The stability of the gel formulation was studied in a stability chamber under accelerated conditions with temperature (40 ± 2 °C) and humidity (75 ± 5 %) following the guidelines of ICH Q1A (R2) guidelines. This study was carried out to investigate the stability of the formulation and predict the shelf life. Gel formulation was subjected to accelerated conditions for a period of 6 months, with analysis points at 0, 3 and 6 months by the HPTLC methods. Physical stability; pH, viscosity, adhesiveness, cohesiveness and chemical stability; assay were the parameters evaluated.

Table 2 QTPP for topical gel

Sr. no.	QTPP		Target	Justification
1	Dosage form		Gel	Topical site of action
2	Amount in jar		30 g	--
3	Route of administration		Topical	Infections occur top layers of skin, local action can be achieved.
4	Dosage strength		10 %	Basing on <i>in-vitro</i> studies
5	pH		6.8-7.2	Compatible with skin
6	Therapeutic use		Anti-microbial	Anti-infective activity
7	Physical attributes	Colour		Colour, odour, appearance and spreadability are not considered as critical, as these are not directly linked to the efficacy and the safety of dosage form
		Odour	No unpleasant odour	
		Appearance	Transparent gel	
		Spreadability	Acceptable spreadability for application	
8	Assay of the optimized extract		VBS : NLT 10 µg/mL	Drug content or Assay is an important parameter for safety and efficacy of the formulation. Material
			COA: NLT 0.21 µg/mL	

		CA: NLT 20 µg/mL	attributes and process parameters can affect the assay of the formulation.
		FA: NLT 18 µg/mL	
9	Container closure system	Round jar	Container closure system
10	Stability	Stable for 6 months	As per ICH Q1A(R2)

Table 3 CQAs for topical gel and their justification

Quality Attributes	Target	Is this CQA?		Justification
Physical Attributes	Colour	Acceptable to the patient	No	Color, shape and appearance are not directly linked to safety and efficacy. Therefore, they are not critical. The target is set to ensure patient acceptability.
	Odour	No unpleasant odour	No	
	Appearance	Acceptable to the patient	No	
Assay	VBS : NLT 10 µg/mL	Yes		Assay is for safety and efficacy of the formulation. It is important for product performance. Material attributes and process parameters can affect the assay of the formulation.
	COA: NLT 0.21 µg/mL			
	CA: NLT 20 µg/mL			
	FA: NLT 18 µg/mL			
Stability	Stable for 6 months	Yes		The gel should be stable, as it affects patient compliance and affects physical and chemical stability and efficacy.
Viscosity	Should have adequate viscosity as a gel.	Yes		Measuring viscosity is crucial for controlling the texture, stability, and application properties of gels.

Table 4 Formulation table for gel

Sr. No.	Ingredient	Strength	Usage
1)	Extract	10 %	Active Ingredient
2)	Carbopol (974)	1 %	Gelling agent
3)	Acacia	1 %	Binder
4)	Propylene glycol	5 %	Solubilizing agent
5)	Preservatives a) Methyl Paraben b) Propyl Paraben	0.1 % 0.1 %	Storage
6)	Water	q.s. up to 100 mL	Solvent
7)	Triethanolamine	q.S	pH balancer

Table 5 Accelerated Stability data

Sr. No.	Test	Results		
		0 month	3 month	6 month
1	Organoleptic evaluation	Has a good appearance and non-gritty.	Has a good appearance and non-gritty.	Has a good appearance and non-gritty
2	Colour	Brown	Brown	Brown
3	Weight variation	-	1%	1.5 %
4	pH	7.05	6.99	6.96
5	Viscosity (cP)	40.169	39.872	39.303
6	Adhesiveness (mJ)	0.94	0.93	0.93
7	Cohesiveness	0.86	0.85	0.83
8	Spreadability	6.3	6.2	6.1
9	Homogeneity	Excellent	Excellent	Excellent

10	Phase separation	No	No	No
11	Assay			
	VBS (µg/mL)	12.16	11.99	11.87
	COA (µg/mL)	2.385	2.35	2.32
	CA (µg/mL)	24.99	24.65	24.40
	FA (µg/mL)	23.99	23.66	23.42

- From the data obtained of stability studies, shelf life for individual marker was calculated. The studies show the rate constant is different for individual marker which makes a difference in their degradation pattern and hence variable shelf lives.
- However, shelf life is never solely dependent on chemical stability of a drug product. Physical stability of a drug product also plays an important role in determining shelf life. In our phytopharmaceutical gel, physical and organoleptic evaluation did not display any change in accelerated conditions whilst in stability studies.
- The Ayurvedic Pharmacopeia of India had a gazette notification released in 2016 on shelf life of Ayurvedic, Unnani, Siddha and homeopath medicines where in Malahar (gel) have been given a shelf life of 3 years.
- Therefore, the expected shelf life of our gel formulation is 3 years.

Achievements with respect to objectives

- Validated HPTLC analytical methods quantified four key phytochemical markers (verbascoside, caffeic acid, coumaric acid, ferulic acid), establishing a robust quality control framework essential for reproducibility and regulatory compliance.
- A 3² full factorial Design of Experiments (DOE) effectively optimized the extraction process, identifying solvent composition and extraction time as critical factors for maximizing marker yield, while ensuring ethanol content remained below antimicrobial thresholds.
- The optimized extract was evaluated for its ethanolic content and *in-vitro* activity against anti-bacterial species. The ethanolic content was found to be less than required for activity and the optimized extract was effective against *S. aureus* and *E. coli*.

- The formulated gel showed favorable physicochemical attributes (viscosity, spreadability, stability) and presents a promising alternative to synthetic topical agents, addressing a pressing need for safer, plant-based therapeutics in the face of rising antimicrobial resistance.
- This study successfully formulated and standardized a topical phytopharmaceutical gel derived from *Kigelia africana* fruit extract, demonstrating significant in-vitro anti-infective activity against *S. aureus* and *E. coli*.

Conclusion

- The accurate, precise and sensitive HPTLC methods verified, developed and validated serve as foundation for estimation of markers in *K. africana* plant. It aids in laying down for chromatographic fingerprinting of the plant.
- An optimized extraction protocol tailored to the specific phytochemical profile of the plant material is essential for producing high-value herbal formulations. The optimization of extraction protocols systematically dissects for the individual and synergistic effects of solvent concentration and extraction time on the yields of critical marker compounds exemplified by *K. africana*.
- Optimizing the extraction process moves it from a preparation step to a strategic lever for enhancing the quality, therapeutic efficacy, and sustainability of herbal products.
- The evaluation of the optimized extract bridged the gap between essential characteristics required for extract as a raw material and the topical dosage form.
- The gel formulation developed, standardized and evaluated showcased a robust, stable and effective formulation against *E. coli* and *S. aureus*. The gel's shelf life calculated through stability studies is expected to be 3 years.

Publications

- 1) Bodiwala Kunjan, Banker Nancy, **Lalwani Rahul**, Shah Mamta, Gajjar Anuradha. Quality control methods for fruit extracts of *Kigelia africana* using high performance thin layer chromatography. Natural Product Research, 39(15), 4409–4417.

- 2) **Lalwani Rahul**, Bodiwala Kunjan, Gajjar Anuradha. High-performance thin-layer chromatography method for the quantitative assessment of caffeic acid, coumaric acid, and ferulic acid in ethanol extracts of *Kigelia africana* fruits. *Journal of Planar Chromatography* **38**, 147–154 (2025).
- 3) Gajjar Anuradha, Gohel Nisha, **Lalwani Rahul**, Bodiwala Kunjan. Instrumental thin-layer chromatography method for the simultaneous estimation of gabapentin and nortriptyline hydrochloride in tablet dosage form. *Journal of Planar Chromatography*. **38**, 611–622 (2025).
- 4) Jani Harshil, Ranch Ketan, **Lalwani Rahul**, Patel Yash, Bodiwala Kunjan, Gajjar Anuradha. Sensitive HPLC Method to Support Pre-Formulation Studies and to Determine Critical Quality Attributes of Therapeutic Contact Lenses Containing Dorzolamide Hydrochloride. *Current Pharmaceutical Analysis*. 2024;20(9):994-1004.

Patents (if any): Yes, a provisional patent has been filed.

Patent no: 20252106956. Date of filing: July 21, 2025.

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