Indian J. Applied & Pure Bio. Vol. 41(2), 762-775 (2026). **ISSN: 0970-2091**

Pharmaceutical and Phytochemical Standardization of *Lakshadi Guggulu* with Special Emphasis on HPTLC Fingerprint Profiling and Its Therapeutic Relevance in Osteoarthritis

[1]Mansi Deore and Vaishali Deshpande[2]

[1,2]Department of Kayachikitsa, Parul Institute of Ayurveda and Research, Parul University, Vadodara - 391760 (India)

Abstract

Lakshadi Guggulu is one of the classical polyherbal Ayurvedic drug formulations that are commonly employed in treating disorders of bone metabolism, healing fractures, or degenerative joint diseases. Despite the common practice of using *Lakshadi Guggulu* in traditional medicine, there is little evidence of employing modern analytical techniques in its standardization.

In the present study, the effort has been made to use High Performance Thin Layer Chromatography (HPTLC) in the standardization of the polyherbal formulation *Lakshadi Guggulu* and correlate the results with various bioactive compounds, which are responsible for the efficacy of the drug in treating certain inflammatory disorders like osteoarthritis.

The methanolic formulation was analyzed at different concentrations using an ultraviolet detector at 254 nm as well as a fluorescent detector at 366 nm.

The consistent chromatographic pattern indicates the uniform presence of resin acids, guggulsterones, phenolic compounds, withanolides, and lipid fractions in the formulation. The dominant appearance of medium-polarity compounds like resinous fractions, fluorescent steroidal lactones, supports evidence of the efficacy of the drug in treating inflammatory diseases like osteoarthritis.

The HPTLC fingerprint serves as a reliable pattern for quality assurance of *Lakshadi Guggulu*. The phytochemicals analyzed in the herbal formula resin acids, guggulsterones, flavonoids, and withanolides have been identified as being related to anti-inflammatory and osteogenic pathways in the pathogenesis of degenerative joint diseases like osteoarthritis.

Key words : *Lakshadi Guggulu*, HPTLC fingerprinting, phytochemical standardization, osteoarthritis, guggulsterones, withanolides.

[1]PG Scholar, [2]Professor

Osteoarthritis is the most common arthritis in the world. Osteoarthritis can be divided into two subgroups: the first one being the primary osteoarthritis and the second one being the secondary osteoarthritis. Osteoarthritis traditionally has been associated with joint pain and loss of function; however, the clinical manifestations of osteoarthritis are highly variable and range from a completely insignificant finding to a devastating disease with a permanently disabling process. Osteoarthritis is a progressive degenerative joint disorder characterized by cartilage erosion, subchondral bone remodelling, synovial inflammation, and chronic pain.[24] Conventional pharmacological management primarily offers symptomatic relief and is often limited by gastrointestinal, cardiovascular, and renal adverse effects with long-term use. This therapeutic gap has renewed interest in traditional formulations with multitargeted mechanisms and favorable safety profiles.

Lakshadi Guggulu, described in classical Ayurvedic texts such as *Bhaishajya Ratnavali, Yogaratnakara,* and *Chakradatta*, is traditionally indicated in *Bhagna* (fractures), Sandhigata Vata, and disorders of bone integrity. The formulation comprises Laksha (Laccifer lacca), Guggulu (Commiphora mukul), Asthishrinkhala (Cissus quadrangularis), Ashwagandha (Withania somnifera), Arjuna (Terminalia arjuna), and Nagabala (Grewia hirsuta). Each constituent contributes distinct pharmacological actions including anti-inflammatory, anabolic, antioxidant, and osteoprotective effects.[19]

Given the inherent chemical complexity of polyherbal formulations, reproducible quality control remains a major challenge. HPTLC fingerprinting offers a rapid, reliable, and cost-effective approach for phytochemical standardization by generating characteristic chromatographic profiles reflective of formulation composition.

Sample Preparation :

A finely powdered sample of Lakshadi Guggulu (5 g) was extracted with 100 mL of methanol using ultrasonication for 15 minutes. The extract was cooled, filtered, and used as the test solution for HPTLC analysis.

Chromatographic Conditions :

Chromatography was performed on precoated silica gel 60 F_{254} HPTLC plates (10 × 10 cm). Samples were applied as 6 mm bands using a CAMAG Linomat 5 applicator. Plate development was carried out up to 8 cm in a saturated twin-trough chamber using chloroform: methanol: formic acid (8:2:0.5 v/v/v) as the mobile phase.

Detection and Documentation :

Developed plates were scanned using a CAMAG TLC Scanner under:

- 254 nm for UV-absorbing compounds.
- 366 nm for fluorescent constituents

Densitometric evaluation was performed using winCATS 4 software. Sample volumes of 5.0, 10.0, 15.0, and 20.0 µL were analyzed to assess consistency and concentration-dependent resolution.

HPTLC Fingerprint at 254 nm :

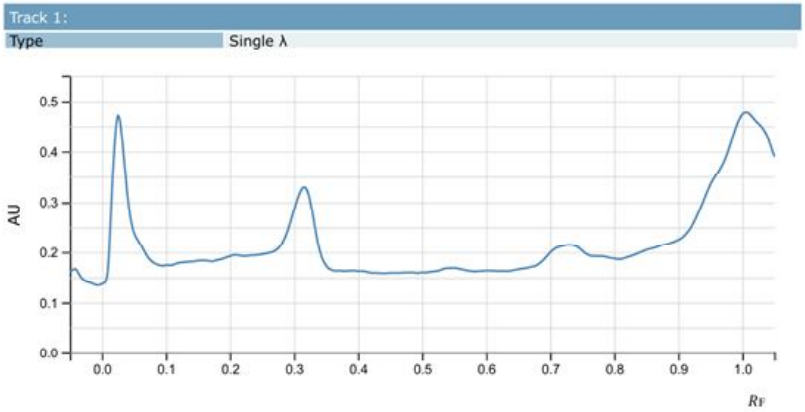


Fig. 1. Track 1 @ 254 nm

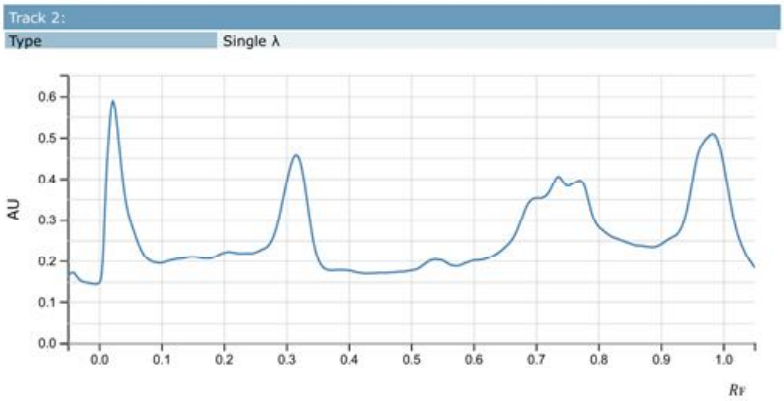


Fig. 2. Track 2 @ 254 nm

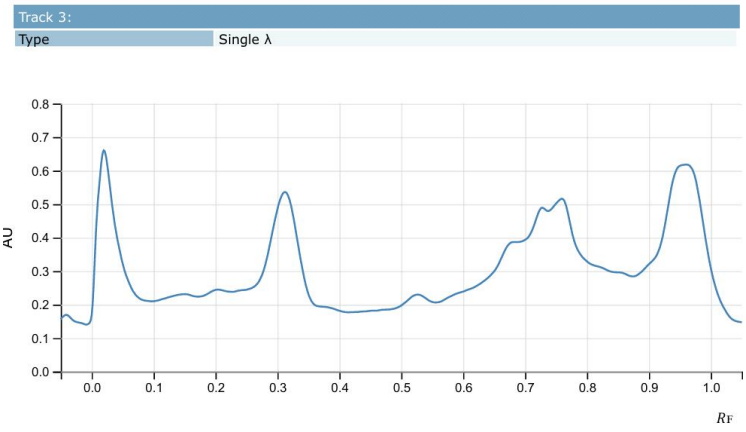


Fig. 3. Track 3 @ 254 nm

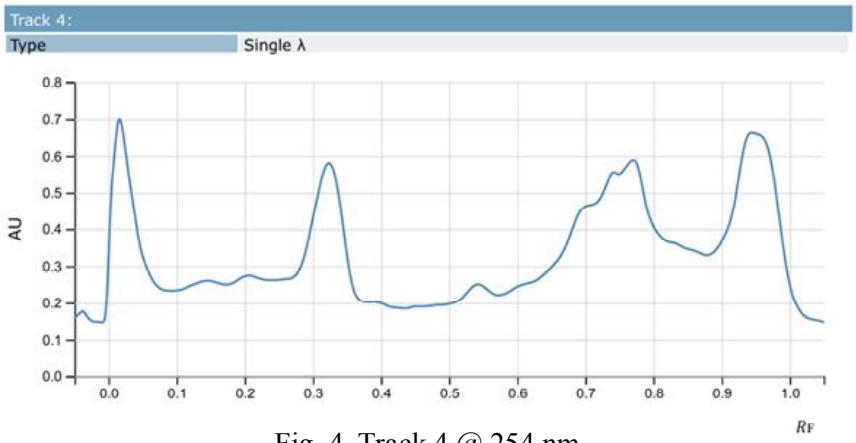
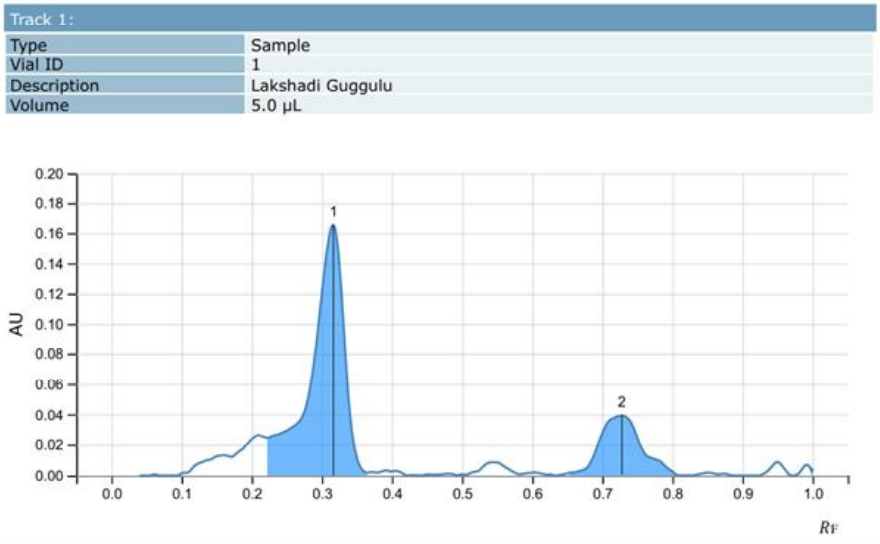


Fig. 4. Track 4 @ 254 nm

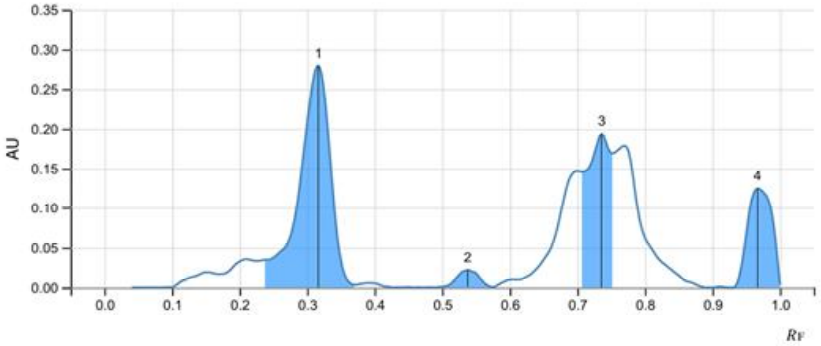
At 254 nm, Lakshadi Guggulu exhibited well-resolved peaks across all concentrations. A dominant band consistently appeared in the Rf range 0.36–0.38, contributing up to 76% of total peak area at lower concentrations, indicating the presence of major UV-absorbing resinous constituents. Additional bands were observed in the Rf ranges 0.55–0.58, 0.73–0.79, and 0.99–1.00, reflecting a complex mixture of phenolics, sterol derivatives, and lipid fractions.



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.222	0.0245	0.317	0.1653	80.80	0.364	0.0018	0.00886	76.48
2	0.651	0.0016	0.728	0.0393	19.20	0.810	0.0000	0.00272	23.52

Fig. 5. Track 1 @ 254 nm

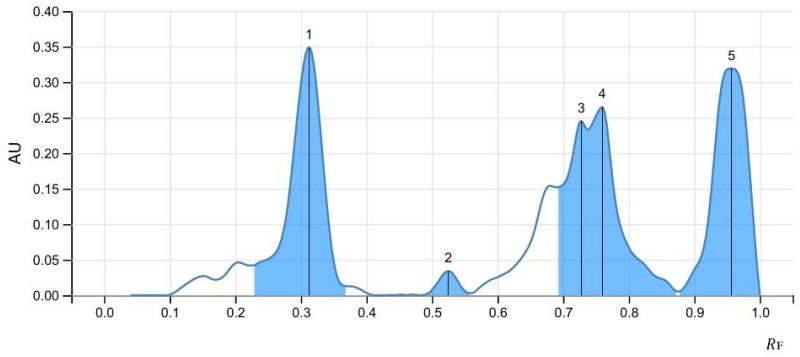
Track 2:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	10.0 µL



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.235	0.0346	0.317	0.2783	44.98	0.371	0.0034	0.01482	52.02
2	0.489	0.0000	0.537	0.0219	3.53	0.574	0.0005	0.00083	2.93
3	0.707	0.1459	0.736	0.1936	31.30	0.751	0.1691	0.00755	26.49
4	0.931	0.0000	0.967	0.1248	20.18	1.000	0.0033	0.00529	18.56

Fig. 6. Track 2 @ 254 nm

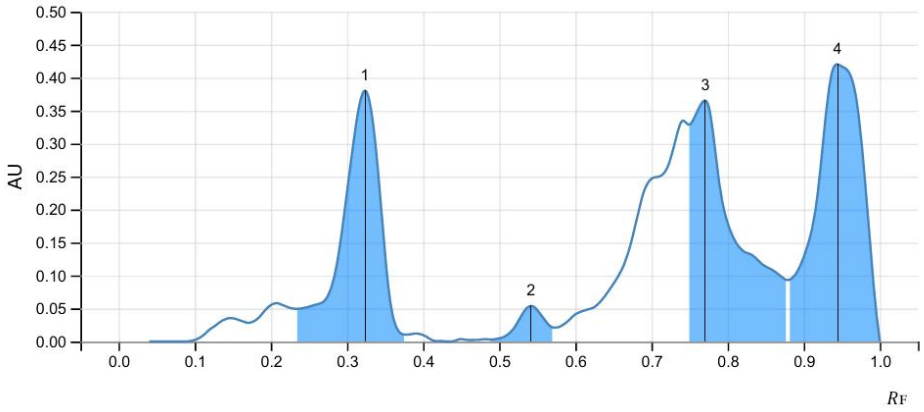
Track 3:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	15.0 µL



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.226	0.0419	0.313	0.3487	28.79	0.375	0.0124	0.02004	31.21
2	0.482	0.0000	0.525	0.0339	2.80	0.558	0.0028	0.00118	1.83
3	0.690	0.1519	0.728	0.2451	20.24	0.738	0.2331	0.00929	14.46
4	0.738	0.2331	0.760	0.2645	21.84	0.875	0.0053	0.01405	21.87
5	0.876	0.0052	0.957	0.3191	26.34	1.000	0.0003	0.01967	30.62

Fig. 7. Track 3 @ 254 nm

Track 4:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	20.0 µL



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.233	0.0493	0.324	0.3801	31.18	0.378	0.0104	0.02185	27.55
2	0.489	0.0029	0.542	0.0538	4.41	0.572	0.0209	0.00226	2.85
3	0.750	0.3288	0.771	0.3650	29.95	0.878	0.0931	0.02506	31.59
4	0.879	0.0930	0.944	0.4200	34.46	1.000	0.0002	0.03014	38.00

Fig. 8. Track 4 @ 254 nm

HPTLC Fingerprint at 366 nm :

Track 1:	
Type	Single λ

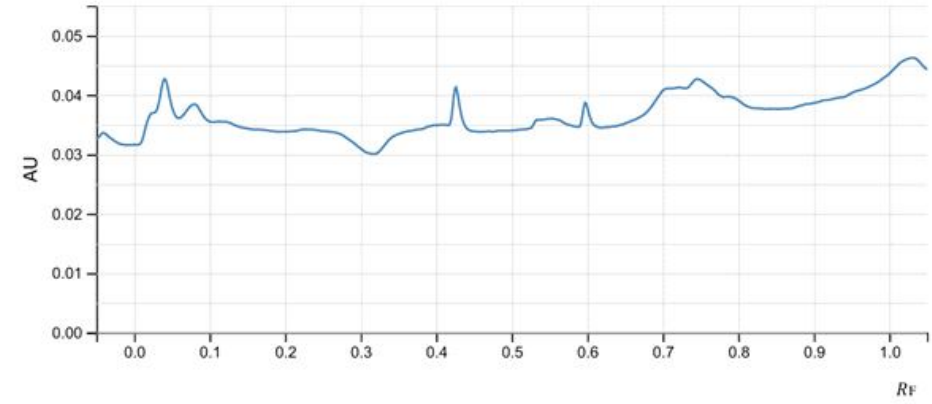


Fig. 9. Track 1 @ 254 nm

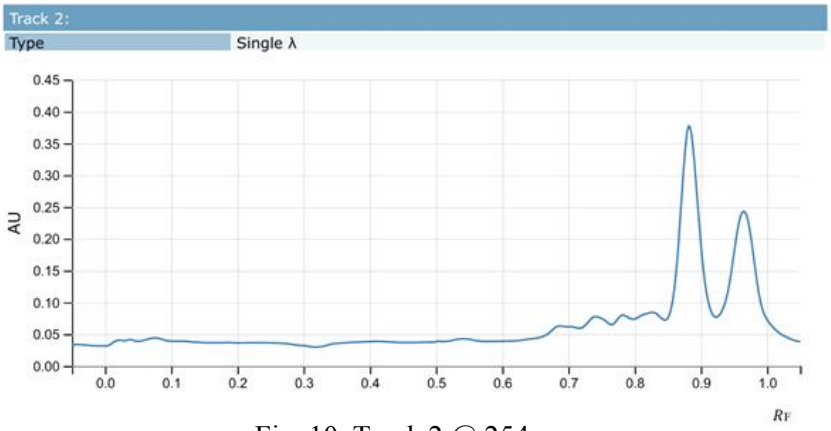


Fig. 10. Track 2 @ 254 nm

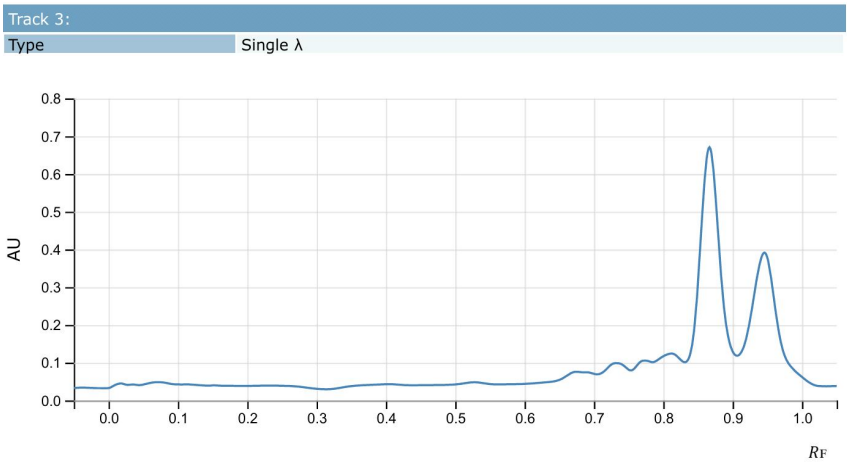


Fig. 11 Track 3 @ 254 nm

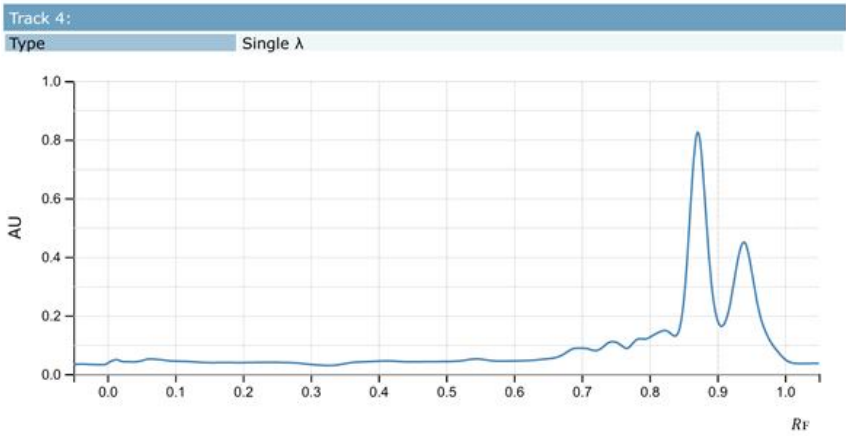


Fig. 12. Track 4 @ 254 nm

Track 1:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	5.0 µL

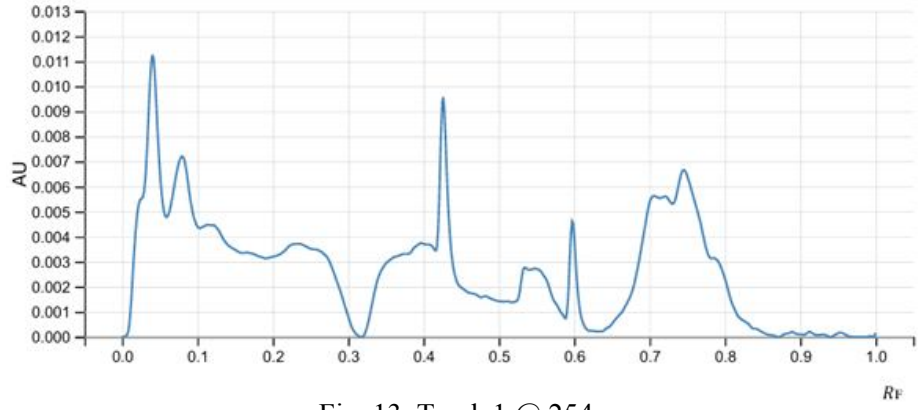
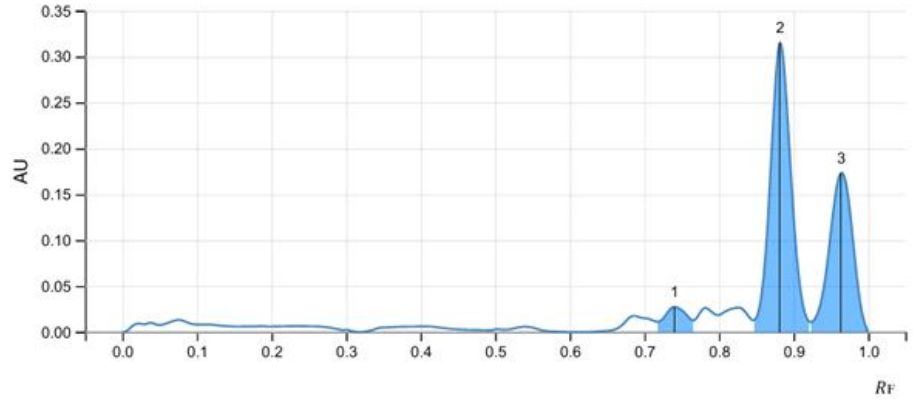


Fig. 13. Track 1 @ 254 nm

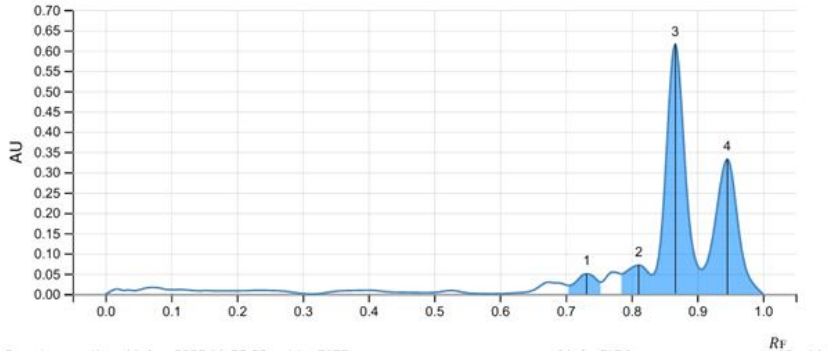
Track 2:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	10.0 µL



Peak	Start		Max			End		Area	
#	R_F	H	R_F	H	%	R_F	H	A	%
1	0.717	0.0112	0.740	0.0274	5.33	0.765	0.0127	0.00097	5.58
2	0.846	0.0128	0.882	0.3148	61.08	0.922	0.0108	0.01010	58.15
3	0.924	0.0107	0.964	0.1731	33.60	1.000	0.0001	0.00630	36.27

Fig. 14. Track 2 @ 254 nm

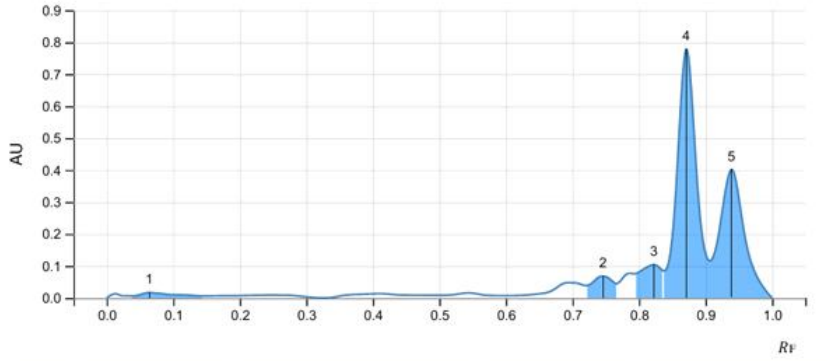
Track 3:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	15.0 µL



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.704	0.0211	0.732	0.0496	4.65	0.753	0.0293	0.00183	4.73
2	0.785	0.0495	0.811	0.0712	6.67	0.831	0.0473	0.00280	7.22
3	0.831	0.0473	0.867	0.6151	57.59	0.906	0.0610	0.02069	53.32
4	0.907	0.0608	0.946	0.3321	31.10	1.000	0.0001	0.01348	34.74

Fig. 15. Track 3 @ 254 nm

Track 4:	
Type	Sample
Vial ID	1
Description	Lakshadi Guggulu
Volume	20.0 µL



Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.037	0.0059	0.064	0.0165	1.20	0.149	0.0059	0.00117	2.30
2	0.721	0.0385	0.746	0.0681	4.97	0.765	0.0451	0.00249	4.91
3	0.793	0.0758	0.822	0.1042	7.61	0.836	0.0854	0.00398	7.85
4	0.838	0.0847	0.871	0.7786	56.89	0.906	0.1163	0.02583	50.96
5	0.907	0.1160	0.939	0.4014	29.33	1.000	0.0000	0.01722	33.98

Fig. 16. Track 4 @ 254 nm

Fluorescence detection at 366 nm revealed prominent peaks in the Rf range 0.81–0.92, accounting for more than 50% of total area at higher concentrations. These fluorescent bands are characteristic of steroidal lactones and withanolides. Minor fluorescent signals at lower Rf values indicated the presence of low-molecular-weight phenolics and organic acids.

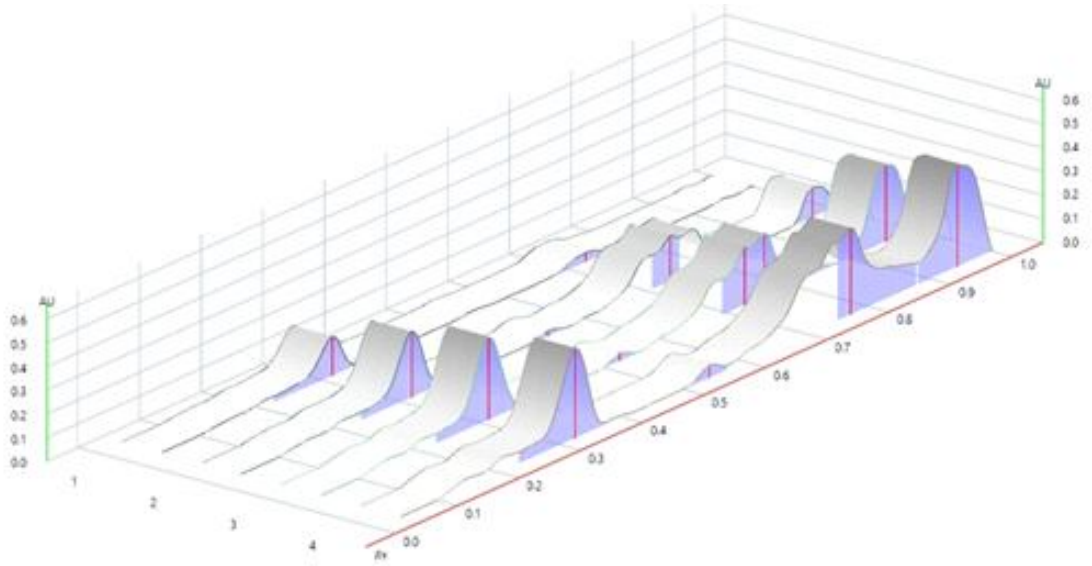


Fig. 17. Result peaks of HPTLC of *Lakshadi Guggulu*

The reproducibility of peak positions and relative areas across concentrations confirms formulation consistency and analytical robustness.

One of the inherent challenges that exist in the standardization of polyherbal preparations like Ayurvedic formulations is the analytical complexity. In the present study, HPTLC fingerprint profiling of Lakshadi Guggulu showed that there is consistency in the chromatographic profile at different concentrations of the formulation. The distribution of peaks is meaningful in understanding the phyto constitution and pharmacological basis of classical use of Lakshadi Guggulu in the management of degenerative musculoskeletal diseases like osteo.

The low-Rf zone (0.14-0.15) was identified as a low portion (~2-3% area), comprising low molecular mass phenolic compounds and organic acids. Although the latter are of a relatively low quantitative nature, these compounds are well acknowledged with regard to their antioxidant buffering potential. Low-polarity phenolic compounds, from sources such as plant resins and barks, with a similar chemical nature to the above-mentioned compounds, were reported to express supportive effects with regard to the process of detoxification, as well as the scavenging of free radicals, which may indirect influence the

inflammatory cascade of chronic joint diseases.[15,21]

The major chromatographic region of interest, *i.e.*, Rf 0.36 - 0.38, was found to account for 27 - 76% of the total area at various concentrations of the formulation. This has thus identified the major bioactive zone of the concern for the formulation. The chromatographic region of interest thus ascribes to the migration pattern of various active constituents of Gugsulsterones, Resin Acids, and other related phenolic terpenoids as identified in the essential components of both Commiphora mukul and Laksha resin. Guggulsterones have been proved to exhibit potent anti-inflammatory activity along with lipid modulatory activity, which can be addressed by the inhibition of NF-κB signaling pathways and suppression of the pathways of both COX-2 and LOX enzymes. These pathways thus relate directly with the pathology of osteoarthritis, which follows a state of low-grade inflammation and cartilage degradation.[2,16,25]

The Rf range of 0.55–0.58, though contributing only 2–3% of the chromatogram, holds considerable pharmacological importance. This Rf range has been attributed to flavonoids and phenolic glycosides, primarily consisting of Cissus quadrangularis, also known as *Asthishringhala*, and *Terminalia arjuna*. These compounds have been found to induce osteoblast proliferation, increase collagen synthesis, and induce mineralization, thus facilitating bone repair and regeneration. Research studies have established the fracture-healing and osteogenic potential of Cissus quadrangularis, validating its inclusion in formulations targeting skeletal disorders.[3,13]

Table-1. Interpretation of HPTLC findings with already available evidence

Rf Range	Observed Area %	Probable Phytochemical I Class	Likely Plant Source(s)	Probable Pharmacological Role	Citations
0.14–0.15	~2–3%	Low-MW phenolics, organic acids	Minor polar fractions from Laksha, trace	Mild antioxidant, base matrix; contributes to detoxification Guggulu components	[6,20]
0.36–0.38	27–76% (Dominant band)	Resin acids, guggulsterones, phenolic terpenoids	Guggulu, Laksha resin	Major anti-inflammatory, anti-arthritic, lipid-modulatory activity (chondroprotective, COX/LOX modulation)	1,17,11
0.55–0.58	~2–3%	Flavonoid glycosides, phenolic glycosides	Asthishrin-ghala, Arjuna	Osteogenic stimulation, antioxidant support, bone healing	[4,5]
0.73–0.79	5–26%	Tannins, oligomeric phenolics, sterol	Arjuna, Nagabala,	Cardioprotective, tissue repair, collagen	[14,13, 12,26]

		glycosides	Ashwagandha	modulation, anti-inflammatory	
0.81–0.92	3–58% (higher under 366 nm)	Withanolides, steroidal lactones, guggulsterones	Ashwagandha, Guggulu	Adaptogenic, anabolic, anti-stress, immunomodulatory effects	[11,12,9]
0.99–1.00	18–38%	Triglycerides, long-chain fatty acids, sterols	Laksha waxes, lipid fraction of Guggulu/ Asthishringhala	Enhances absorption, deep tissue penetration	[7,12]

The middle Rf region 0.73–0.79 showed a moderate but variable peak area 5–26% corresponding to tannins, oligomeric phenolics, and sterol glycosides contributed presumably by *Terminalia arjuna*, *Grewia hirsuta (Nagabala*), and *Withania somnifera*. Tannins and triterpenoidal phenolics possess well-documented astringent, anti-inflammatory, and wound-healing properties. These compounds are also known for modulating collagen cross-linking and stability of the extracellular matrix, which is of direct relevance in osteoarthritis where degradation of cartilage matrix is a hallmark feature. The cardioactive profile of Terminalia arjuna-based phenolics may also be beneficial in elderly osteoarthritis patients with associated cardiovascular risk factors.[22,23]

The most striking feature of the HPTLC profile was the high-Rf fluorescent zone 0.81–0.92, which was particularly intense under 366 nm detection up to 58% area, representative of withanolides and steroidal lactones, chiefly from *Withania somnifera*, with overlap of guggulsterone fractions. Withanolides are reported to be adaptogenic, immunomodulatory, and anti-stress agents, which are reported to dampen oxidative stress and inflammatory intermediates. In the context of osteoarthritis, these actions may additively or synergistically contribute to symptom amelioration functional outcome, and tolerance to chronic disease stressors. Extensive reviews have documented the multi-system pharmacology of withanolides and thereby confer strong biological rationale to their role in this formulation.[10,8]

The Rf zone 0.99–1.00 represented a significant non-polar fraction (18–38%) contributed by triglycerides, long-chain fatty acids, and sterols of the Laksha waxes, and lipid-rich fractions of Guggulu and Asthishringhala. While lipid fractions are traditionally considered to be pharmacologically inactive, recent evidence does prove that they often enhance solubility, membrane permeability, and deeper tissue penetration of the co-administered phytoconstituents. According to Ayurvedic pharmaceutics, such a lipid matrix could be traditionally recognized to provide a *Yogavahi* (bio-carrying) action that can extend the therapeutic efficacy into deeper tissues.[18,27]

Therapeutic and Clinical Relevance :

The pharmaceutical standardization

demonstrated in this study provides analytical support to the ongoing randomized double-blind placebo-controlled clinical trial evaluating Lakshadi Guggulu with physiotherapy in knee osteoarthritis. The presence of bioactive phytochemical classes with known anti-inflammatory and osteogenic effects strengthens the biological plausibility of observed clinical outcomes such as pain reduction, improved joint mobility, and reduced reliance on rescue analgesics.

HPTLC fingerprinting of Lakshadi Guggulu establishes a characteristic and reproducible phytochemical profile that can serve as a reference standard for quality control. The detected bands correspond to pharmacologically relevant constituents that collectively explain the formulation's therapeutic efficacy in degenerative joint disorders. Integrating classical Ayurvedic formulations with modern analytical validation not only enhances scientific credibility but also supports their rational clinical application in chronic musculoskeletal diseases.

References :

1. Chaudhary AK, and N. Singh, N. Verma (2011). *J Ethnopharmacol. 135*(1): 1–8.
2. Deng R, and TJ. Chow (2007). *Am J Cardiovasc Drugs. 7*(3): 215-227.
3. Dhanapal S, S Duraisamy and P. Arumugam (2012). *J Ethnopharmacol. 142*(3): 590-597.
4. Dhanapal S, I Joe, and PA. Kurup (2012). *J Ethnopharmacol. 142*(3): 590–595.
5. Kapoor LD. (2001). Handbook of Ayurvedic Medicinal Plants: Herbal Reference Library. Boca Raton: CRC Press.
6. Kokate CK, AP Purohit, and SB. Gokhale (2015). Pharmacognosy. 51st ed. Pune: Nirali Prakashan.
7. Kokate CK, AP Purohit, and SB. Gokhale (2015). Pharmacognosy. 51st ed. Pune: Nirali Prakashan.
8. Kulkarni SK, and A. Dhir (2008). *Prog Neuropsychopharmacol Biol Psychiatry. 32*(5): 1093-1105.
9. Kulkarni SK, and A. Dhir (2008). *Indian J Exp Biol. 46*(7): 509–515.
10. Mishra LC, BB Singh, and S. Dagenais (2000). *Altern Med Rev. 5*(4): 334-346.
11. Mishra LC. (2004). Scientific Basis for the Therapeutic Use of Withania somnifera (Ashwagandha): A Review. Boca Raton: CRC Press.
12. Nadkarni KM. (1976). Indian Materia Medica. 3rd ed. Mumbai: Popular Prakashan.
13. Nair TG, RV Geetha and M. Kumaraswamy (2014). *Pharmacogn Rev. 8*(16): 133–138.
14. Nair TG, and RV. Geetha (2014). *Pharmacogn Rev. 8*(16): 133-138.
15. Pandey KB, and SI. Rizvi (2009). *Oxid Med Cell Longev. 2*(5): 270-278. PMID: 20716914 doi:10.4161/oxim.2.5.9498
16. Patil SB, DK Bagewadi and GM. Sreenivasa (2012). *J Ethnopharmacol. 142*(1): 267-272. doi:10.1016/j.jep.2012.04.048
17. Patil SB, BS Simant, and AR. Shirode (2012). *Phytother Res. 26*(11): 1599–1607
18. Porter CJH, NL Trevaskis, and WN. Charman (2007). *Nat Rev Drug Discov. 6*(3): 231-248.
19. Rajoria K, SK Singh, RS Sharma, and SN. (2010). *Ayu. 31*(1): 80-87. doi:10.4103/0974-8520.68192
20. Rastogi RP, and BN. Mehrotra (1980). Compendium of Indian Medicinal Plants. Vols. 1–5. Lucknow: Central Drug Research

Institute; 1995.
21. Rice-Evans C, N Miller, and G. Paganga (1997). *Trends Plant Sci. 2*(4): 152-159. doi:10.1016/S1360-1385(97)01018-2
22. Sabu MC, and R. Kuttan (2002). *J Ethnopharmacol. 81*(2): 155-160.
23. Scalbert A. (1991). *Phytochemistry. 30*(12): 3875-3883. doi:10.1016/0031-9422(91)83426-L
24. Sen R, and JA. Hurley (2025). Osteoarthritis. [Updated 2023 Feb 20]. In: Stat Pearls [Internet]. Treasure Island (FL): StatPearls Publishing; Available from: https://www.ncbi.nlm.nih.gov/books/NBK482326/
25. Singh BB, LC Mishra, and SP Vinjamury, *et al*. (2003). *Altern Ther Health Med. 9*(3): 74-79.
26. Singh S, A Sharma, and S. Gupta (2013). *Indian J Nat Prod Resour. 4*(2): 184–190.
27. Thatte U, and S. Bhalerao (2008). *J Ayurveda Integr Med. 1*(2): 1-6.