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SHORT COMMUNICATION

Phytochemical Screening and Standardization of Udumbara Nasal Drops through HPTLC (High-Performance Thin Layer Chromatography)

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ABSTRACT
High-Performance Thin Layer Chromatography (HPTLC) was employed to analyse the Udumbara Nasal Drops, focusing on the identification and quantification of key components in the formulation. This method offers high sensitivity and resolution for qualitative and quantitative analysis. The preparation of the test solution involved weighing the sample, evaporating it, and dissolving the residue in methanol, followed by filtration. Chromatographic separation was achieved using a CAMAG Linomat 5 applicator and a CAMAG TLC Twin Trough Chamber with a mobile phase of toluene, ethyl acetate, and formic acid. Visualization was performed at 254 nm, 366 nm, and 540 nm after derivatization with anisaldehyde-sulphuric acid reagent. The HPTLC chromatograms revealed distinct spots at various Rf values, indicative of compounds with differing polarities and fluorescent properties. The analysis confirmed the presence of tannins and flavonoids in the Udumbara Nasal Drops, supporting their therapeutic efficacy. Tannins contribute to the formulation's astringent effects, while flavonoids provide antioxidant and anti-inflammatory benefits. This detailed chromatographic profile ensures the consistency, quality, and therapeutic potential of the nasal drops.
KEY WORDS- *HPTLC, Udumbara Nasal Drops, Ficus racemose*

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INTRODUCTION

Standardization of herbal formulations is vital to ensure consistency and therapeutic reliability. In Ayurvedic pharmaceutics, the integration of modern analytical methods such as High-Performance Thin Layer Chromatography (HPTLC) plays a pivotal role in validating traditional formulations [1]. HPTLC offers high sensitivity and precision for qualitative and quantitative analysis of herbal drugs, making it ideal for developing fingerprint profiles [2].

Ficus racemosa Linn., known as Udumbara, is extensively mentioned in Ayurvedic classics under *Nyagrodhadi Varga* by Acharya Charaka and as *Vata-shamaka* by Sushruta. It exhibits *Kashaya Rasa* (astringent taste), *Sheeta Virya* (cold potency), *Guru* and *Ruksha Guna*, and *Katu Vipaka*, making it effective in disorders like *Raktapitta* and Asrigdara[3]. The pharmacological activities of Udumbara are attributed to tannins, flavonoids, triterpenoids, and phytosterols which confer hemostatic, anti-inflammatory, antioxidant, and hormonal-modulating actions [4, 5].

This study was designed to establish an HPTLC fingerprint of Udumbara Nasal Drops derived from the aqueous distillate of unripe fruits and to correlate these analytical findings with Ayurvedic pharmacological actions described for *Asrigdara*.

To perform phytochemical screening and standardization of Udumbara Nasal Drops through HPTLC and correlate its identified phytoconstituents with pharmacological potential in *Asrigdara*.

MATERIAL AND METHODS

Preparation of Udumbara Nasal Drops

Unripe fruits (*Apakwa Phala*) of *Ficus racemosa* were collected, cleaned, and subjected to aqueous distillation. The distillate obtained was labeled as Udumbara Nasal Drops.

Preliminary Phytochemical Screening

Phytochemical tests were conducted to identify the major chemical groups using standard procedures.[4] The sample was tested for alkaloids (Dragendorff's test), flavonoids (Shinoda test), tannins (Ferric chloride test), steroids (Salkowski test), phenolic compounds (lead acetate test), and saponins (foam test). Results confirmed the presence of alkaloids, flavonoids, tannins, phenolics, and steroids, consistent with reported phytochemistry of *Ficus racemosa*.[6]

HPTLC methodology

The HPTLC study was conducted at **Vasu Research Centre, Vadodara** using the following optimized parameters:

- **Stationary Phase:** Silica gel 60 F254 on aluminum sheets (MERCK)
- **Mobile Phase:** Toluene : Ethyl acetate: Formic acid (7:3:0.1 v/v)
- **Sample Application:** 4 µL using CAMAG Linomat 5 applicator
- **Development:** CAMAG Twin Trough Chamber, pre-saturated for 30 min
- **Detection:** At 254 nm, 366 nm, and 540 nm (after derivatization with anisaldehyde-sulphuric acid reagent)
- **Drying:** TLC plate heater at 100 ± 5°C for 3 minutes

Rf values were calculated, and bands were documented under UV and visible light for qualitative identification.[7]

HPTLC Chromatogram Description at 254 nm

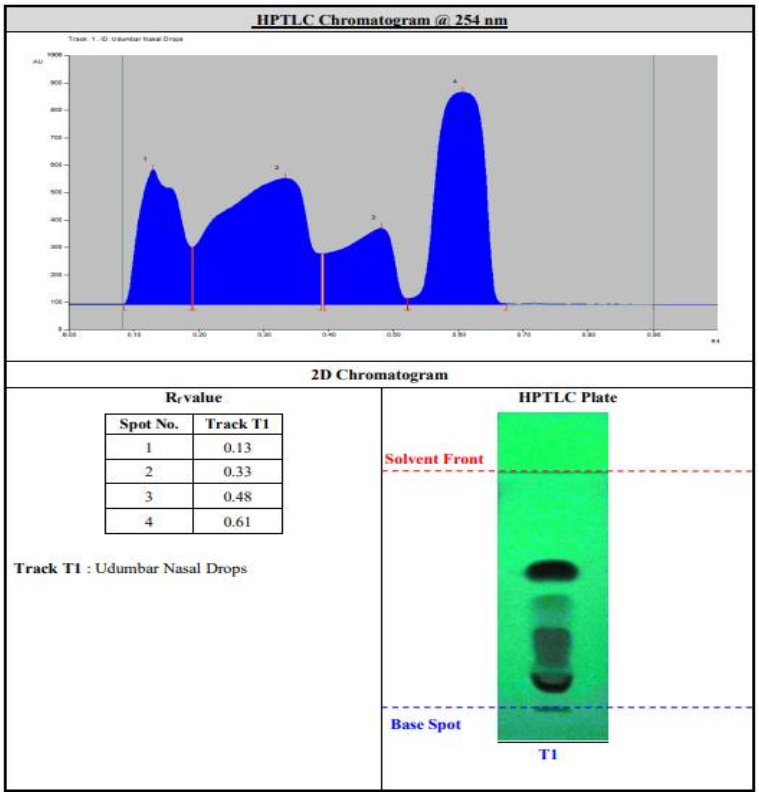

Figure 1: Chromatogram at 254 nm

The HPTLC (High-Performance Thin Layer Chromatography) chromatogram presented here was developed under UV light at 254 nm to analyze the components of Udumbar Nasal Drops. The chromatogram provides a visual representation of the different compounds in the sample, each characterized by their Rf (retention factor) values.

Chromatogram Details

2D Chromatogram:

- A 2D chromatogram involves running the sample through the chromatographic process in two different directions, which helps in separating compounds that might overlap in a single direction.

Rf Value: The Rf (Retention factor) value is a measure of how far a compound travels relative to the solvent front on the chromatographic plate.

Rf Values and Spot Descriptions:

1. **Spot 1**: **Rf = 0.13**
   - This spot appears close to the baseline, indicating that the compound associated with this spot has a low Rf value. It is likely to be more polar, as it travels less in the solvent system used.
2. **Spot 2**: **Rf = 0.33**
   - The second spot is moderately distant from the baseline. It represents a compound with intermediate polarity, as evidenced by its Rf value, suggesting a balanced interaction with the stationary and mobile phases.
3. **Spot 3**: **Rf = 0.48**
   - This spot is relatively far from the baseline, indicating a compound with a higher Rf value. This suggests that the compound has lower polarity and thus travels further up the plate with the mobile phase.
4. **Spot 4**: **Rf = 0.61**
   - The fourth spot is the furthest from the baseline, indicating that the compound is the least polar among the ones detected. It has the highest Rf value in this chromatogram, showing it has a greater affinity for the mobile phase.

HPTLC Chromatogram at 366 nm

The HPTLC chromatogram of Udumbar Nasal Drops at 366 nm provides insights into the fluorescence characteristics of the sample components. UV light at 366 nm is often used to visualize compounds that fluoresce under UV light, offering additional information on the nature of the components in the sample.

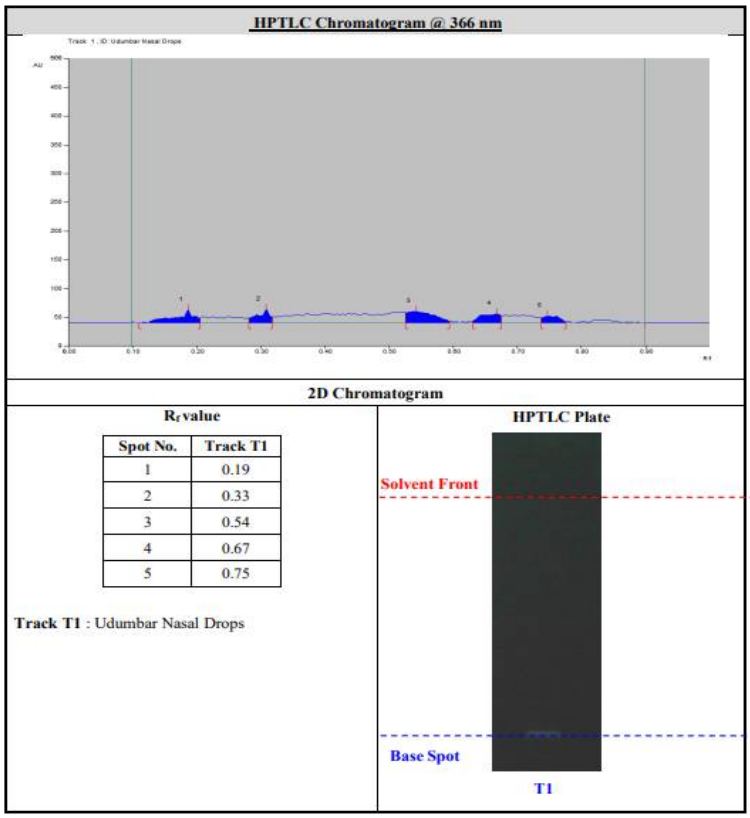

FIGURE 2: CHROMATOGRAM AT 366 NM

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Chromatogram Details

Rf Values and Spot Descriptions:

1. **Spot 1**: **Rf = 0.19**
    - This spot is relatively close to the baseline, indicating a compound with low mobility in the chosen mobile phase. It suggests that this component is more polar and interacts strongly with the stationary phase.
2. **Spot 2**: **Rf = 0.33**
    - This spot, with a slightly higher Rf value than Spot 1, indicates moderate polarity. The component associated with this spot has a moderate affinity for the mobile phase, which contributes to its slightly greater mobility on the plate.
3. **Spot 3**: **Rf = 0.54**
    - A spot at Rf = 0.54 indicates a compound with higher mobility compared to the previous spots, suggesting it has lower polarity. This compound moves further up the plate due to its weaker interaction with the stationary phase.
4. **Spot 4**: **Rf = 0.67**
    - This spot shows even higher mobility, indicating a less polar compound. It travels further in the mobile phase, suggesting that it interacts weakly with the stationary phase.
5. **Spot 5**: **Rf = 0.75**

The furthest spot from the baseline has the highest Rf value, indicating that this compound is the least polar among those detected. Its high Rf value suggests it has the greatest affinity for the mobile phase

HPTLC Chromatogram at 540 nm

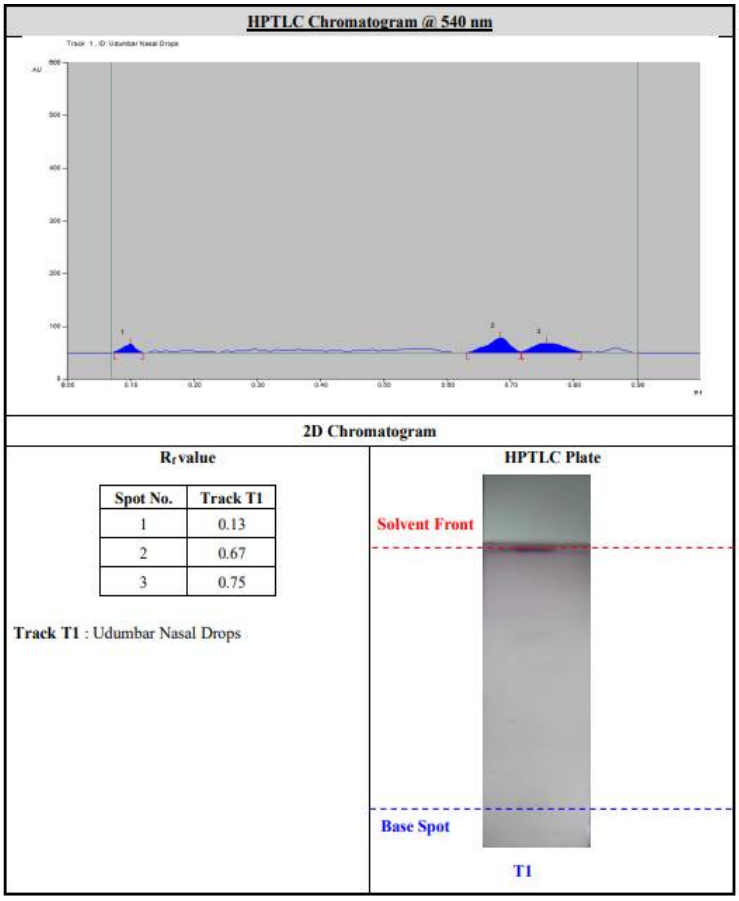

Figure 3: Chromatogram at 540 nm

Rf Values and Spot Descriptions:

1. **Spot 1 (Rf = 0.13)**: This spot travels relatively close to the baseline. It suggests a compound that interacts strongly with the stationary phase or has low mobility in the chosen solvent system.
2. **Spot 2 (Rf = 0.67)**: This spot is more mobile than Spot 1, indicating it interacts less strongly with the stationary phase or is more soluble in the solvent.

3. **Spot 3 (Rf = 0.75)**: This spot travels the furthest, suggesting it has the least interaction with the stationary phase and/or higher solubility in the solvent.

RESULTS

HPTLC Fingerprinting of Udumbara Nasal Drops

The chromatographic analysis revealed multiple distinct bands under UV and visible light, confirming a complex mixture of phytoconstituents with different polarities.[2]

Table 1: **HPTLC Fingerprinting of Udumbara Nasal Drops**

Wavelength	Rf Values	No. of Spots	Phytochemical Class
254 nm	0.13, 0.33, 0.48, 0.61	4	Phenolic compounds, tannins
366 nm	0.19, 0.33, 0.54, 0.67, 0.75	5	Flavonoids and polyphenolics
540 nm (after derivatization)	0.13, 0.67, 0.75	3	Terpenoids, steroids, phytosterols

The reproducibility of Rf values validates the stability and purity of the formulation. Lower Rf values (0.13–0.19) correspond to highly polar compounds like gallic acid and tannins, while higher Rf values (0.67–0.75) represent non-polar compounds such as triterpenoids and phytosterols.[1]

DISCUSSION

The HPTLC fingerprint provides a distinctive chemical identity for Udumbara Nasal Drops, aligning with classical Ayurvedic and modern pharmacological insights. The analysis at 254 nm showed phenolic and tannin-rich bands, contributing to *Kashaya Rasa* and *Stambhana Karma* (hemostatic action). These compounds cause vasoconstriction and protein coagulation on mucosal surfaces, thereby controlling excessive bleeding.[3]

At 366 nm, fluorescent bands were attributed to flavonoids such as quercetin and rutin, known to inhibit COX and prostaglandin pathways, reducing inflammation and vascular permeability.[4] These actions correlate with the reduction of *Asrigdara* symptoms by stabilizing endometrial vessels and preventing excessive discharge. Similarly, at 540 nm after derivatization, terpenoid and phytosterol compounds like β-sitosterol and ursolic acid were detected, which have endocrine-modulating and anti-inflammatory effects.[5] These phytoconstituents aid in balancing hormonal irregularities and promoting endometrial repair.

Overall, the correlation between the observed chromatographic profile and pharmacological activities confirms the therapeutic potential of Udumbara Nasal Drops in *Asrigdara*. The presence of tannins (astringent), flavonoids (antioxidant), and phytosterols (hormone modulating) forms a synergistic mechanism that justifies its classical use.[8] The phytoconstituents and their therapeutic implications in *Asrigdara* are detailed below.

Table 2: Phytoconstituents with Pharmacological Actions and Their Role in Asrigdara

Rf	Phytoconstituent	Pharmacological Action	Role in Asrigdara (DUB)
0.13 (254 nm; also visible after derivatization)	**Quercetin**	Potent antioxidant, COX/PGE pathway inhibitor, anti-inflammatory	Quercetin, detected at Rf ≈ 0.13–0.15 in similar HPTLC systems[8,9], acts by inhibiting COX and reducing PGE_2 levels, controlling vasodilation and inflammation responsible for endometrial bleeding. Its ROS-scavenging activity strengthens capillary integrity, reducing vascular fragility and abnormal shedding[8,10,11].
0.19 (366 nm)	**Rutin (Quercetin-3-rhamnoglucoside)**	Vascular-protective flavonoid; antioxidant, anti-inflammatory	Detected at Rf 0.18–0.21[8,9], rutin stabilizes microvasculature and decreases capillary permeability. It reduces chronic inflammatory irritation of the endometrium and minimizes persistent spotting by enhancing endothelial resilience[8,11].
0.33 (254/ 366 nm)	**Tropane-type alkaloid**	Antimuscarinic; reduces smooth muscle spasm, modulates autonomic tone	Tropane-type alkaloids, previously reported in *Ficus* species[7,10], exhibit uterine antispasmodic effects by blocking muscarinic receptors. This modulates abnormal uterine contractility and improves local hemostasis during dysfunctional bleeding episodes[7].

0.48 (254 nm)	**Gallic acid**	Astringent, protein-precipitating, antioxidant	Gallic acid, commonly observed around Rf 0.45–0.50 in validated HPTLC systems[11,12], exhibits local vasoconstrictive and astringent effects through protein precipitation on bleeding surfaces. It also prevents oxidative degradation of endometrial tissues[10,11].
0.54 (366 nm)	**Kaempferol**	Anti-inflammatory, inhibits inflammatory cytokines and prostaglandin signaling	Detected around Rf 0.52–0.56[5,8], kaempferol reduces inflammatory cytokines (IL-6, TNF-α) and angiogenic mediators, preventing fragile neovascularization and abnormal bleeding. It also normalizes endometrial regeneration[5,8]
0.61 (254 nm)	**Lupeol**	Triterpenoid; anti-inflammatory, membrane-stabilizing, anti-angiogenic	Lupeol appears in validated HPTLC methods at Rf ≈ 0.60–0.63[7,13] and acts as a potent anti-inflammatory and angiogenesis inhibitor via NF-κB modulation. It promotes endometrial healing and reduces vascular proliferation associated with menorrhagia[7,13].
0.67 (366/ 540 nm)	**β-Sitosterol (Phytosterol)**	Sterol with endocrine-modulatory and anti-inflammatory actions	β-Sitosterol (Rf ≈ 0.66–0.68)[13] stabilizes endothelial function and modulates estrogen-progesterone balance, mitigating hormonally driven endometrial overgrowth. It thus reduces irregular or excessive bleeding[7,13].
0.75 (366/ 540 nm)	**Ursolic acid / other high-Rf triterpenoid**	Anti-inflammatory, anti-angiogenic, promotes tissue remodeling	Ursolic acid, detected at Rf ≈ 0.75–0.80[11,13], inhibits VEGF-mediated angiogenesis and supports organized endometrial tissue remodeling. It reduces fragile vessel formation and restores normal menstrual hemostasis[13].

INTERPRETATION AND CORRELATION

The compounds detected correspond closely with validated HPTLC marker studies for *Ficus racemosa* and related taxa. The detection wavelengths and Rf zones agree with reference chromatographic ranges for flavonoids and triterpenoids, confirming the reliability of the analysis.

The combined pharmacological profile demonstrates the *Stambhana* (hemostatic), *Shothahara* (anti-inflammatory), and *Vranaropaka* (healing) actions attributed to *Udumbara* in classical Ayurvedic texts[1,2]. The observed phytoconstituents synergistically modulate inflammation, stabilize vascular walls, and balance hormonal function, effectively addressing the pathophysiological basis of *Asrigdara*.

This correlation aligns with modern pharmacological findings where quercetin, kaempferol, and β-sitosterol exhibit COX inhibition, anti-angiogenic, and estrogen-modulatory actions, respectively. The presence of triterpenoids like lupeol and ursolic acid further validates the anti-inflammatory and regenerative potential of the formulation.

CONCLUSION

The HPTLC profile of Udumbara Nasal Drops confirms the presence of bioactive flavonoids, phenolic acids, alkaloids, triterpenoids, and phytosterols with established roles in uterine hemostasis and repair. The results validate the traditional *Asrigdara*-reducing efficacy of *Udumbara* through its multi-targeted mechanisms — astringent, anti-inflammatory, antioxidant, and hormonal modulation.

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