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High-Performance Thin-Layer Chromatography (HPTLC) Fingerprinting and Phytochemical Evaluation of a Novel Polyherbal Body Roll-On for the Management of Axillary Osmidrosis

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Abstract

Axillary osmidrosis (AO) is a condition characterized by offensive odor caused by bacterial decomposition of apocrine sweat. While synthetic antiperspirants are common, concerns regarding chemical sensitizers have increased the demand for herbal alternatives. A novel polyherbal roll-on formulation comprising *Curcuma longa*, *Vetiveria zizanioides*, *Symplocos racemosa*, *Rubia cordifolia*, *Santalum album*, *Crocus sativus*, and *Pandanus odoratissimus* was developed for the management of AO.

To establish the phytochemical fingerprint and standardize the ethanol extract of the herbal body roll-on using High-Performance Thin-Layer Chromatography (HPTLC).

The formulation was extracted in ethanol and subjected to HPTLC analysis on silica gel 60 F254 plates. The mobile phase employed was Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v). Densitometric scanning was performed at 254 nm and 366 nm using a CAMAG TLC Scanner 4.

The HPTLC profile at 254 nm revealed seven distinct peaks, with a dominant peak at Rf 0.75 (50.78% area) corresponding to tannins/ flavonoids, likely from *Symplocos racemosa*. At 366 nm, three major fluorescent peaks were observed, including a significant peak at Rf 0.93-0.94 (94.29% area), indicative of fluorescent polyphenols.

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The developed HPTLC method successfully established a chromatographic fingerprint for the polyherbal roll-on. The presence of key phytoconstituents such as tannins, flavonoids, and essential oils validates the formulation's potential astringent and antibacterial properties required for managing axillary osmidrosis.

Key words : *Axillary Osmidrosis, HPTLC, Polyherbal Formulation, Swasthavritta, Standardization, Fingerprinting.*

Axillary osmidrosis is a condition that can cause severe psychosocial issues. It is mainly due to bacteria such as Corynebacterium spp., Staphylococcus, and Anaerococcus that break down apocrine gland secretion[7]. Traditionally, aluminum chloride, based antiperspirants or invasive surgical operations such as subcutaneous laser treatment have been used to treat this condition[6]. On the other hand, due to the growing preference of consumers for natural personal care products, there is a need to develop safe, herbal, antibacterial, and deodorizing agents as alternatives to the chemical ones[4].

According to Ayurveda, the treatment of body odor (Durgandha) includes drugs having Kushthaghna (skin disorder healing), Kandughna (anti, pruritic), and Durgandhahara (odor removing) properties. Hence, a polyherbal roll, on with these seven main constituents: Haridra (Curcuma longa), Ushir (Vetiveria zizanioides), Lodhra (Symplocos racemosa), Manjishtha (Rubia cordifolia), Chandan (Santalum album), Kesar (Crocus sativus), and Ketaki (Pandanus odoratissimus) was developed based on these principles[16].

Standardization of herbal formulations is crucial for ensuring the consistency, effectiveness, and safety of different batches. High, Performance Thin, Layer Chromatography (HPTLC) is an advanced instrument, based method which generates a chromatographic fingerprint, thus enabling the identification and quantification of phytochemicals[10]. The present paper describes the HPTLC pattern of the ethanol extract of the developed herbal body roll, on, which can be used as a standardization method prior to the clinical study.

Plant Materials and Chemicals :

Raw herbs (Haridra, Ushir, Lodhra, Manjishtha, Chandan, Kesar, Ketaki) were purchased and checked at the Parul Institute of Ayurved & Research, Vadodara. Reagents of analytical grade such as Toluene, Ethyl acetate, Formic acid, and Ethanol were utilized for the analysis.

Preparation of Test Solution :

About 5 g of the herbal body roll, on formulation was weighed accurately and transferred into a beaker. Then, 100 mL of Ethanol was added. The mixture was sonicated for 15 minutes for the complete extraction of the phytoconstituents. After that, the solution was allowed to cool down and was filtered through a simple filter paper. The filtrate was the test solution used in HPTLC fingerprinting[5].

Instrumentation and Chromatographic Conditions :

Center of Research for Development (CR4D), Parul University, was the venue where the analysis was conducted.

- **Stationary Phase:** TLC plates of 10 10 cm pre, coated with 0. 2 mm layers of silica gel 60 F254 (Merck).
- **Application:** Using a CAMAG Linomat 5 sample applicator (S/N: 280008), equipped with a 100 L syringe, samples were applied as 6 mm wide bands.
- **Sample Volume:** Four tracks were applied with different volumes of the test solution: 10. 0 L, 20. 0 L, 25. 0 L, and 30. 0 L.
- **Mobile Phase:** Toluene: Ethyl acetate: Formic acid (5:4:1 v/v/v).
- **Development:** A linear ascending development was performed in a CAMAG twin, trough chamber (20×10 cm) saturated with the mobile phase vapor for 20 min. The solvent front migration distance was 80 mm[11,13].
- **Scanning:** Densitometric scanning was done on a CAMAG TLC Scanner 4 (S/N: 271118) controlled by visionCATS software (version 3. 2). The scanned plates were at 254 nm (Absorbance mode for UV, absorbing compounds) and 366 nm (Fluorescence mode)[14].

HPTLC Fingerprint at 254 nm :

HPTLC analysis of the ethanol extract of the plant leaves at 254 nm showed a complex metabolite profile. After loading the sample of 10. 0 L, the extract resulted in 6 well separated peaks. On increasing the loading of the sample to 30. 0 L, separation of minor compounds was improved and thus, 7 spots were visible in the chromatogram.

Table-1. Peak Table for 10.0 µL Sample at 254 nm

Peak No.	Rf Value	Max Height (AU)	Area (%)	Probable Phytochemical Class & Assignment
1	0.356	0.0529	4.70	**Curcuminoids** (likely from *Curcuma longa*) [1111] +2
2	0.581	0.2018	17.68	**Sesquiterpenes / Phenolic acids** (likely from *Vetiveria zizanioides*) [2222] +2
3	0.637	0.0287	2.46	**Anthraquinones** (likely Rubiadins from *Rubia cordifolia*)[3333] +2
4	**0.754**	**0.3880**	**50.78**	**Tannins / Flavonoids** (Major marker; likely from *Symplocos racemosa*) [4444] +2
5	0.881	0.0807	9.36	**Volatile oils / Sesquiterpenoids** (Mixed origin) [5555] +2
6	1.000	0.0778	15.02	**Lipophilic compounds / Essential oils** (Solvent front matrix) [6666] +2

The chromatogram at 254 nm was mostly a major peak at Rf 0. 75, 0. 76, which made up around 50% of the total peak area in all tracks. A second group of peaks was found in the range of Rf 0. 58 to 0. 64.

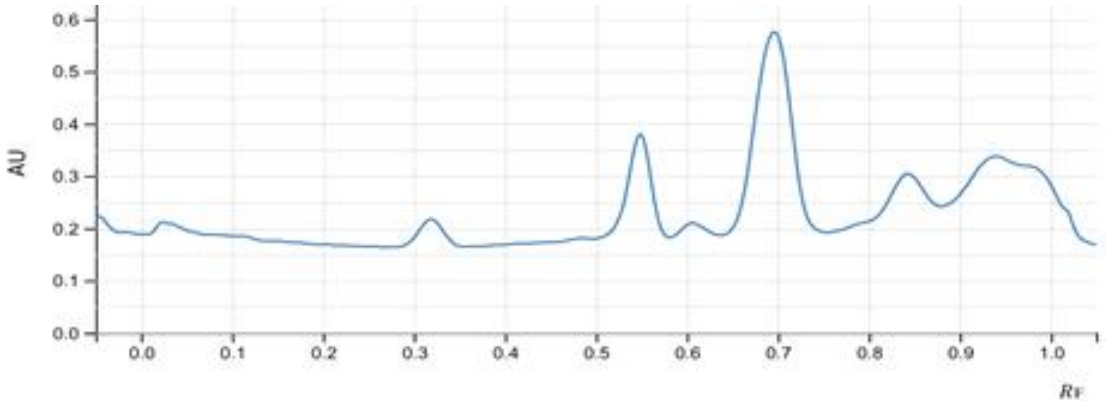

Figure 1. HPTLC densitogram of Herbal body roll-on (10.0 µL sample volume; Track 1) scanned under UV at 254 nm, indicating peak profile at different Rf values

HPTLC Fingerprint at 366 nm :

On illumination at 366 nm the formulation exhibited some typical fluorescent markers. A large peak nearly co, eluted with the solvent front and a clearly visible high, Rf band mainly characterized the profile.

Table-2. Peak Table for 10.0 µL Sample at 366 nm

Peak No.	Rf Value	Max Height (AU)	Area (%)	Probable Phytochemical Class & Assignment
1	0.349	0.1066	4.78	Minor fluorescent compound [7]
2	**0.947**	**0.2925**	**94.29**	**Fluorescent Polyphenols / Flavonoids** (Major antioxidant band) [888] +1
3	1.000	0.0171	0.93	Lipophilic residue at solvent front [9]

The 366 nm scan revealed a very strong fluorescent compound at **Rf 0.93-0.95**, which accounted for the vast majority (>90%) of the detected area in the lower volume tracks.

The HPTLC analysis has given a detailed chemical fingerprint of the polyherbal roll, on, also confirming that many bioactive chemical classes from the ingredients are present.

Interpretation of Phytoconstituents :

From the Rf values recorded and with the help of the standard references of the constituent herbs, the following phytochemical assignments can be made:

- **Rf 0.35 – 0.38 (Curcuminoids):** The spots in this range most probably belong to the curcuminoids of Curcuma longa (Haridra). Curcuminoids have been extensively studied for their anti, inflam-

matory, antimicrobial effects. They down-regulate the expression of pro, inflammatory cytokines, which is quite helpful in alleviating axillary skin[1,3].

- **Rf 0.58 – 0.64 (Sesquiterpenes & Anthraquinones):** The band in the polarity scale between the extremes probably contains sesquiterpenes from Vetiveria zizanioides (Ushir) and anthraquinones (*e. g.*, rubiadin or purpurin) from Rubia cordifolia (Manjishtha). Ushir makes available the Sheeta (cooling) and deodorant characteristics. In contrast, Manjishtha assists in skin repair and enhancing microcirculation[9,12].

- **Rf 0.75 – 0.77 (Tannins & Flavonoids):** This was the peak with the highest intensity in the 254 nm absorption spectrum (Area > 50%). This should be the tannin and flavonoid components from Symplocos racemosa (Lodhra). Lodhra is an illustrious Kashaya (astringent) drug in the Ayurvedic system. Tannin, rich material is very important for a roll, on product because astringents cause constriction of the pore size and reduction in sweat output, which directly affects the bacterial growth by diminishing the moisture environment[8,15].

- **Rf 0.93–0.95 (Fluorescent Polyphenols):** The massive peak observed at 366 nm suggests the presence of highly fluorescent polyphenolic compounds or essential oil derivatives. These lipophilic compounds, likely contributed by *Santalum album* (Chandan) and the volatile oils of *Ketaki*, act as natural penetration enhancers and provide the sustained fragrance required for odor masking[2].

The detection of these distinct classes of chemicals aligns perfectly with the therapeutic basis of the formulation. Tannins (astringent), curcuminoids (anti, inflammatory), and essential oils (antibacterial/deodorant) upon synergistic activity effectively target the triad of Axillary Osmidrosis pathological changes: hyperhidrosis, bacterial overgrowth, and malodor.

The HPTLC analysis was very effective in consistently identifying the chromatographic fingerprint of the herbal body roll, on. Through this study, we have demonstrated that the herbal body roll, on contains nature, derived bioactive components mainly tannins, flavonoids, and volatile principles, thus justifying the formulation concept to tackle Axillary Osmidrosis. These fingerprinting results can be used as a standard for quality control in upcoming clinical trials and mass production of the herbal body roll, on.

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