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COMPARATIVE CHROMATOGRAPHIC PROFILING OF DIFFERENT PHARMACEUTICAL PREPARATIONS MADE FROM *ROOTS* OF *PRISNIPARNI (DESMODIUM GANGETICUM* (L) DC) USING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Introduction: Ayurvedic literature emphasizes diverse pharmaceutical processes, including variations in solvents, heating techniques, and preparation media to optimize therapeutic benefits. Interestingly, the same plant part is often used in different dosage forms based on the disease context, indicating a strategic approach to enhance efficacy. *Prisniparni* is documented in classical Ayurvedic literature as a versatile drug, administered as formulations such as *kwatha*, *ksheerapaka*, and *churna*. The physicochemical properties of plant secondary metabolites influence disease modulation, and different preparation methods may cause structural modifications in these metabolites. However, such transformations remain underexplored. **Aim:** This study aimed to evaluate the phytochemical variation in *Prisniparni* rootwhen subjected to four preparation methods: methanolic extract, decoction (*kwatha*), traditional *ksheerapaka*, and a modified *ksheerapaka* made by mixing dried decoction powder with skimmed milk powder. *Desmodium gangeticum* (L.) DC. (Leguminosae), the accepted source of *Prisniparni* in Kerala, was used as the source drug in this study.

Materials and Methods: High-Performance Thin Layer Chromatography was employed to generate fingerprint profiles. Chromatographic separation was analyzed by comparing Rf values and visualizing bands under UV 254 nm and UV 366 nm. **Results:** Each formulation exhibited a unique set of bands, indicating substantial variation in phytochemical content. While some bands were common across preparations, others were formulation-specific, suggesting chemical transformation during the preparation process. **Conclusions:** The study demonstrates that pharmaceutical processing significantly alters the composition of chemical constituents in *Prisniparni* root. These findings support the Ayurvedic principle that preparation methods influence therapeutic potential. Further pharmacological investigations are essential to correlate these chemical differences with bioactivity and therapeutic relevance.

Key-words: *Desmodium gangeticum*; *Prisniparni*; Pharmaceutical processing; Dosage forms; Secondary metabolites; Phytochemical transformation.

1. INTRODUCTION: Ayurveda utilizes a wide variety of medicinal plants either as *ekala oushadhas* (single drug) or as *oushadha yogas* (formulations). These *yogas* were developed by *Acharyas* (ancient Ayurvedic scholars) combining suitable plants or plant parts for particular disease conditions through their practical experience. *Acharyas* also introduced different *Kalpanas* (preparation techniques) for making medicines using different solvents, different medium of cooking, heating methods, soaking methods, etc. with a purpose of getting maximum therapeutic potential. Interestingly, they recommended the use of same medicinal plant parts in different pharmaceutical form in different disease conditions to increase the therapeutic efficacy.[1]*Prisniparni* is one such plant described in *Ayurveda* classical text books and root is recognized as the officinal part. Although some ambiguity exists regarding its botanical source, *Desmodium gangeticum* (L.) DC. (Leguminosae) is the accepted species in Kerala and was therefore used as the source drug in this study.[2] It is traditionally used in the management of *jwara* (fever),*vata rogas*(diseases due to vitiated vata),*Kasa* (cough),*Unmada*(insanity),*atisara* (diarrhoea) etc.[3]It is an ingredient of many formulations and its use as a milk-based decoction is recommended for the treatment of *Vatika hridroga* (heart disease due to vitiated *vata*).[4]Additionally, its *kwatha* (decoction)is prescribed in *rakta arsas* (bleeding piles)[5],while *churna* (powder) along with *mamsarasa* (meat soup) is advised in cases of *asthi bhanga* (bone fractures).[6]

The therapeutic efficacy of raw drugs and prepared formulations may be attributed to the diverse chemical constituents they contain, each or in combination, possessing specific biological activities. Structural alterations of these compounds during preparation could contribute to the enhanced therapeutic effects of the final formulation. The nature of the raw materials and the methods of preparation are two critical factors influencing these structural changes. Often, Ayurvedic formulations are prepared by prolonged heating of various raw drugs in different media, such as water, milk etc. Such processes can lead to chemical modifications resulting from the co-extraction and interaction of multiple bioactive compounds.[7]For instance, in the preparation of *Ksheerapaka* of *Prisniparni* (Milk-based decoction), plant root is boiled with milk and water in a ratio of 1:8:32 until the mixture reduces to the volume of milk.[8]Apart from the extraction of both hydrophilic and lipophilic substances, there might be a chance of chemical transformation by the interaction of phytoconstituents of plant with milk constituents while heating. For the preparation of *kwatha* from the same plant, the root is boiled with water in a 1:16 ratio until the volume reduces to one-eighth, which facilitates the extraction of predominantly hydrophilic compounds.[9]In both situations only heat stable phytoconstituents are available in the final product. In recent times, plant extracts have gained greater popularity over such traditional preparations due to factors such as better palatability, shelf life and ease of administration.

Pharmaceutical modifications of primary preparations, such as *kwatha* tablets, are

also increasingly common. Although these dosage forms are more convenient to carry and consume with good shelf life, the extent of their therapeutic equivalence to classical preparations remains uncertain. To address this dilemma, modern analytical techniques play a crucial role. Comparative chemical profiling of formulations with raw drugs is one of the most reliable methods to understand the chemical transformations due to different methods of preparation. High-performance thin-layer chromatography (HPTLC) is a strong widely used analytical tool for the comparative profiling of formulations with their raw drugs.[10]

In the present study, HPTLC analysis was performed on *Desmodium gangeticum* (L) DC root to analyze its chemical profile in four different preparations: methanolic extract, *kwatha*, conventionally prepared *ksheerapaka*, and a modified *ksheerapaka* form prepared by combining dried decoction powder with skimmed milk powder. The identified small molecules based on Rf value were subsequently compared to elucidate the chemical transformations taking place during the preparation process.

2.MATERIALS AND METHODS.

Precoated silica gel 60 F 254 HPTLC plate (20.0 x 15.0 cm) and all analytical grade solvents and chemicals were procured from E. Merck KGaA. Whole plant of *Prisniparni* (*Desmodium gangeticum* (L.) DC) collected from Tripunithura area of Ernakulam district of Kerala was botanically identified by a qualified taxonomist from the Department of Botany, St. Alberts College, Ernakulam. A voucher specimen was deposited in the registered herbarium of the same institution.

Figure 1 *Desmodium gangeticum* (L.) DC. Herbarium specimen

2.1Preparation of Samples. Samples were prepared at Drug Standardization unit of Department of Dravyaguna, Government Ayurveda College, Tripunithura in standard conditions. Four different samples were prepared from dry root of *Desmodium gangeticum* (L.) DC) and details are listed in Table1.

Table 1: Preparation methods of *Desmodium gangeticum* roots samples for HPTLC analysis

Sample	Method of preparation
Sample 1 Methanol extract	50 gm of coarsely powdered root were subjected to cold maceration with 500 ml of methanol (10:1, v/w) at room temperature (25 ± 2 °C) for 72 hours with occasional shaking. The mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The residue was remacerated with 250 ml of methanol for 48 hours under identical conditions. Both filtrates were pooled for further processing.
Sample 2 kwatha (decoction)	48 gm of crushed root were taken in an earthen pot with 768 ml of water, following classical *kwatha* preparation methods described in *Śārṅgadhara Saṃhitā* (Madhyama Khanda).[11]The mixture was boiled on a medium flame until reduced to 96 ml.
Sample 3 *Ksheerapaka*	Raw cow's milk from an approved farm outlet in Tripunithura, Ernakulam (Kerala), and dried crushed root of identified *Desmodium gangeticum* (L.) DC. were used for the preparation. *Ksheerapaka* was prepared following the classical method described in *Śārṅgadhara Saṃhitā* (Madhyama Khanda),[12]using one part of the drug with eight parts milk and thirty-two parts water. The mixture was boiled over medium heat in an earthen pot until reduced to the original quantity of milk.
Sample 4 Dried powder of *kwatha* + skimmed milk powder.	*Kwatha* was prepared using the same method as Sample 2. The water content was evaporated using a heating mantle followed by gentle heating in a water bath. The resulting semisolid was scraped from the beaker, shade-dried and kept in a desiccator. Then it is placed in a hot air oven at mild temperature for 1.5 hours to ensure complete drying. The dried residue was powdered using a mortar and pestle. Based on the total solid content of cow's milk and classical *Ksheerapaka* references,64 g of skimmed milk powder from an FSSAI-approved company was added to the preparation.

2.3HPTLC Profiling : HPTLC analysis was performed using a CAMAG HPTLC system. Samples were applied on a precoated silica gel 60 F 254 HPTLC plate using a LINOMAT 5 applicator fitted with 100 µl syringe. Samples were applied as 90.0 µl bands with a constant application rate of 150 nl/s. No pre-washing and modification of the plate were done. Chloroform: Ethyl acetate: Formic acid

(5:4:1) was used as mobile phase. Chromatogram was developed in a twin-trough chromatographic chamber (CAMAG) which is preconditioned with mobile phase vapour for 30 min. Developed plate was dried and scanned using a CAMAG TLC Scanner. The bands were visualized using CAMAG visualizer, and the images were captured in white light and 254 nm (short UV) and 366 nm (long UV) wavelengths. Integrated software win CATS Planar Chromatography Manager (CAMAG) was used for data processing and Rf value of bands were recorded.

3.RESULTS: Comparative HPTLC profiling of four different pharmaceutical preparations of *Desmodium gangeticum* (L) DC root were performed. HPTLC profile of all four samples at wavelength of 254 nm are presented in Figure 2. Chemical quenching at UV-254 nm and fluorescence at UV-366 nm have complied, and the data is presented in Table 2 and Table 3 respectively

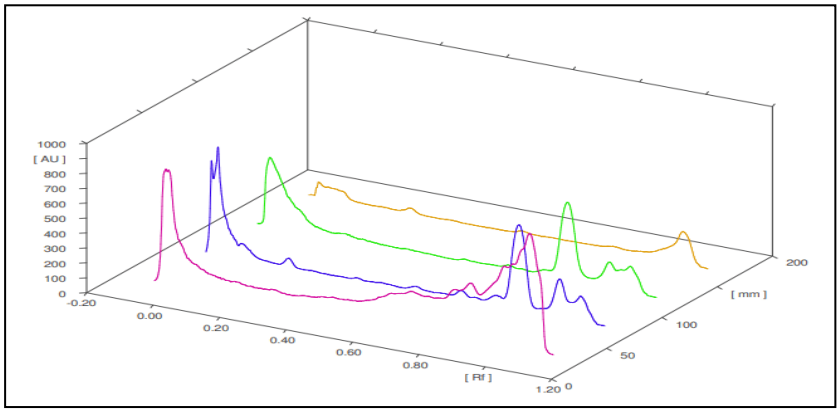

Figure 2: HPTLC Chromatogram of four samples of *Desmodium gangeticum* tracks at wavelength of 254 nm.

This 3D densitogram illustrates the comparative phytochemical fingerprinting of four different preparations of *Desmodium gangeticum* root. The X-axis represents the Retention factor (Rf value), the Y-axis shows absorbance intensity (AU), and the Z-axis corresponds to migration distance along the TLC plate (mm). This figure highlights qualitative differences in the chemical profiles of the four preparations, reflecting the influence of traditional processing methods on chemical constituents. The colour representation is as follows: Methanol extract (Cold maceration method)- Pink; Classical *kwatha* (Aqueous decoction)- Blue; *Ksheerapaka* (Milk-based decoction as per Ayurvedic Texts)- Green; Dried *Kwatha* Powder + Skimmed Milk Powder (Reconstructed *Ksheerapaka*)- Orange

Table 2:Rf values and number of spots detected at wavelength of 254 nm

No. of spots	Methanol extract	*Kwatha*	*Ksheerapaka*	Dried powder of *kwatha* + Skimmed milk powder
1.	-0.06	-0.07	0.08	-0.04
2.	0.02	0.00	0.20	0.04
3.	0.18	0.07	0.55	0.24
4.	0.38	0.18	0.69	0.57
5.	0.43	0.38	0.90	0.83
6.	0.71	0.57	0.99	0.91
7.	0.82	0.70	1.08	1.09
8.	0.88	0.80	_	_
9.	0.97	0.90	_	_
10.	1.10	1.00		
11.	_	1.08	_	_

This table presents the Rf values of various chemical constituents separated by HPTLC at 254 nm for each preparation of *Desmodium gangeticum* (L) D Croot.

Table 3: Rf values and number of spots detected at wavelength of 366 nm

No of spots	Methanol extract	*kwatha*	*Ksheerapaka*	Dried powder of *kwatha* + Skimmed milk powder
1.	-0.06	-0.08	-0.05	-0.03
2.	0.03	-0.06	0.08	0.02
3.	0.02	-0.02	0.18	0.59
4.	0.14	0.00	0.86	0.96
5.	0.18	0.05	0.92	1.09
6.	0.26	0.13	0.99	_
7.	0.49	0.13	1.08	_
8.	0.58	0.45	_	_
9.	0.67	0.61	_	_
10.	0.74	0.82	_	_
11.	0.82	1.08	_	_
12.	0.88	_	_	_
13.	0.94	_	_	_
14.	0.98	_	_	_
15.	1.00	_	_	_
16.	1.10	_	_	_

This table presents the Rf values of various chemical constituents separated by HPTLC at 366 nm for each preparation of *Desmodium gangeticum* (L) DC root. Chromatographic patterns visualized under UV 254nm, UV 366 nm and white light is given in Figure 3, Figure 4 and Figure 5 respectively.

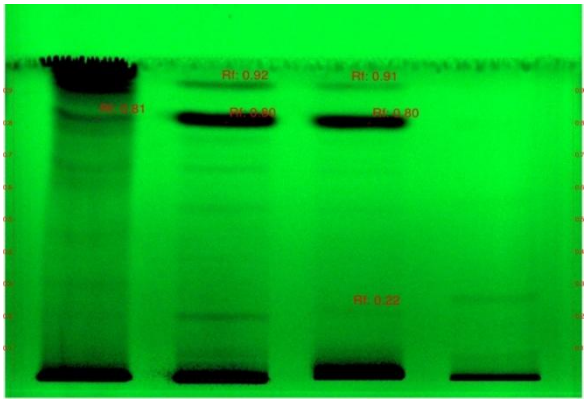

Figure 3: Comparative HPTLC profiling for each preparation of *Desmodium gangeticum* (L) DC root visualized under UV light at 254 nm.

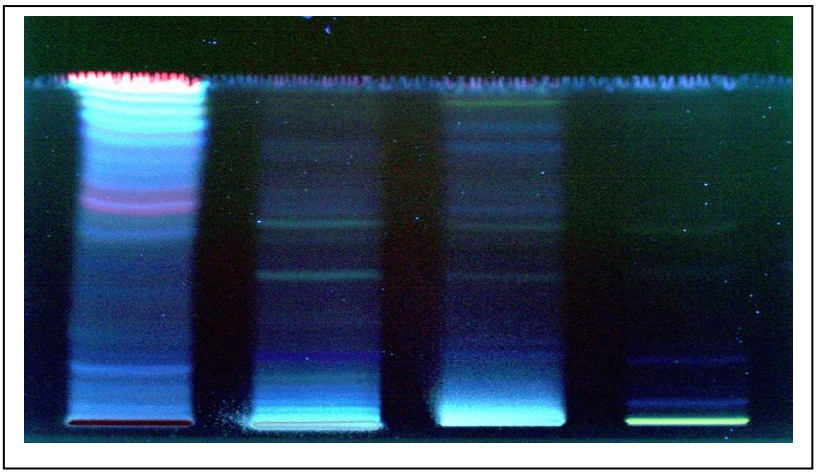

Figure 4: Comparative HPTLC profiling for each preparation of *Desmodium gangeticum* (L) DC root visualized under UV light at 366nm.

Figure 5: Comparative HPTLC profiling for each preparation of *Desmodium gangeticum* (L) DC root under visible light.

4.DISCUSSION: *Desmodium gangeticum* (L.) DC, commonly known as *Prisniparni* in Kerala, isa well-known medicinal plant in Ayurveda. Classical Ayurvedic texts describe the use of *Prisniparni* root as powder, decoction, and milk-based decoction for managing various diseases. The therapeutic potential of such formulations depends heavily on their phytochemical composition, which can be significantly influenced by the preparation method. In this context, HPTLC was employed to compare the chemical fingerprints of four formulations made from *Prisniparni* roots, revealing how different processing techniques affect the presence and distribution of chemical constituents.

Distinct variations in the number and intensity of spots were observed across the samples (from Table 1) indicating the influence of extraction media and processing methods on chemical composition. Among all samples, the methanol extract (sample 1) exhibited the highest number of spots under both wavelengths (10 at 254 nm and 16 at 366 nm) indicating its efficiency in extracting a broad spectrum of chemical constituents, including polar and moderately non-polar compounds. The classical *kwatha* preparation (sample 2) displayed 11 spots at both 254 nm and 366 nm, demonstrating a moderate range of water-soluble components. The *ksheerapaka* (Sample 3) preparation yielded 7 spots at both wavelengths, reflecting the selective extraction of milk-soluble and thermally stable phytoconstituents. The dried powder of *kwatha* combined with skimmed milk powder (Sample 4) showed 7 spots at 254 nm and 5 at 366 nm, suggesting potential compound degradation or matrix effects during drying and reconstitution.

Notably, several overlapping Rf values were observed across samples. At 254 nm, Rf 0.18 and 0.38 were shared between the methanol extract and *kwatha*, while Rf 0.57 was common to *kwatha* and the dried formulation. Rf 1.08 was seen in both *kwatha* and *ksheerapaka*. These shared spots suggest the presence of consistent chemical constituents retained across aqueous and milk-based preparations. At 366 nm, Rf 0.82 appeared in both methanol extract and *kwatha*, Rf 1.08 was shared by *kwatha* and *ksheerapaka*, and Rf 0.02 was common to methanol extract and the dried formulation. Unique peaks at Rf 0.59 and 0.96 in the dried sample at 366 nm, absent in *ksheerapaka*, indicate possible structural alterations during the drying process.

Overall, the findings underscore the impact of extraction medium and preparation technique on the chemical constituent diversity and fluorescence behavior of *Desmodium gangeticum* (L) DC roots. Methanol extraction was the most comprehensive, confirming its broad-spectrum solubilizing efficiency. while traditional formulations like *kwatha* and *ksheerapaka* demonstrated targeted selectivity and lesser number of spots at 366 nm indicating a reduced diversity of UV-active compounds—possibly due to protein binding or transformation during milk-based processing. The dried formulation effectively mimicked the phytochemical traits of both parent preparations, making it a viable reconstructed alternative.

The HPTLC chromatogram under UV 254 nm reveals distinct banding patterns across the analyzed tracks, indicating the presence of multiple chemical constituents. Prominent bands with corresponding Rf values (0.22, 0.80, 0.81, 0.91, and 0.92) indicate the presence of major phytoconstituents across different samples, demonstrating both qualitative similarities and differences in chemical composition.

A strong and consistent band at Rf 0.80 appears in two of the tracks, suggesting the presence of a common compound. High Rf values around 0.91 and 0.92 imply the presence of non-polar compounds in the upper regions of the plate. The intense spot at Rf 0.22 in one of the samples suggests a unique or highly concentrated polar compound. The slight variations in Rf values and band intensities highlight both shared and unique constituents among the samples, reflecting differences in phytochemical profiles that may result from variation in extraction method, solvent polarity etc.

Under UV 366 nm, the HPTLC chromatogram reveals a more vivid and differentiated profile of chemical constituents, compared to UV 254 nm. Multiple fluorescent bands of varying colours—blue, green, pink, and yellow—are evident, each corresponding to different types of compounds, likely including flavonoids, phenolics, and coumarins.[13,14] The presence of sharp and bright bands in certain tracks, especially near the lower and upper regions, suggests a high concentration of specific UV-fluorescent compounds. Notably, some bands are common across the tracks, implying shared constituents, while others are unique, indicating variations in chemical composition between samples. These differences may result from the extraction method or the solvent used, and provide valuable insights for fingerprinting, quality control, and chemotaxonomic evaluation.

The HPTLC profile under visible light reveals comparatively fewer and lighter bands, primarily brownish and yellow in tone. These bands correspond to compounds that are naturally coloured or undergo chromogenic reactions without the need for UV activation. The most prominent bands are visible near the base and mid-region of the tracks, possibly indicating the presence of pigmented phytoconstituents.[15,16] While the overall number of visible bands is lower than those seen under UV 254 nm or 366 nm, this chromatogram is still valuable for detecting compounds with inherent colour. The variation in band intensity and distribution among the tracks further supports differences in the qualitative composition of the tested preparations.

5.CONCLUSION

The comparative HPTLC analysis demonstrated clear formulation-dependent variation in the phytochemical profiles of *Desmodium gangeticum* root preparations. Methanol extract exhibited the greatest constituent diversity, whereas *kwatha, ksheerapaka*, and the dried formulation showed more selective chemical patterns consistent with their extraction processes. Both shared and distinct Rf values across wavelengths confirm that processing influences the retention, loss, or transformation of key phytoconstituents.

Overall, these findings highlight the critical role of preparation method in defining chemical composition and provide a basis for future pharmacological correlation and standardization efforts.

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