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## A Comparative Analysis of *Churna* and *Ksheerapaka* of Dried Root of *Prishniparni- Desmodium Gangeticum (Linn.) DC.* through High Performance Thin Layer Chromatography.

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### ABSTRACT:

High Performance thin layer chromatography has become a potent analytical technique in the process of drug discovery. It serves as a tool for identification, authentication and quality control of herbal drugs due to its reliability and simplicity. *Prishniparni -Desmodium gangeticum* (Linn.) DC. is a significant and well-known drug expoundly discussed in the *Ayurvedic* classics. It is an erect diffusely branched undershrub with enriched therapeutic potential. The plant root is rich in flavonoids, iso flavonoids, cardiac glycosides, pterocarpan, alkaloids and steroids which accounts for its multiple beneficial action in the therapeutics. It has strong antioxidant and cardioprotective properties. The *churna* (powder) and *ksheerapaka* (milk decoction) of dried root of *Prishniparni* was subjected to High Performance thin layer chromatography and comparative analysis of different peaks were obtained. The results suggest that change in phytochemical constituents and their quantity occur on changing the Kalpana (dosage form) from *churna* (powder) to *ksheerapaka* (milk decoction).

**Key words:** High Performance thin layer chromatography, *churna*, *ksheerapaka*, *Prishniparni*

### INTRODUCTION

High Performance thin layer chromatography is one among the reliable and highly sophisticated analytical techniques for qualitative, quantitative and semi-quantitative analysis of herbal drugs. It serves as the most potent quality control for herbal medicine due to its consistency and reliability, thus looked upon as a tool for identification, authentication

and quality control. It works on the principle of absorption. HPTLC can be used to check adulteration and ensure purity of the drug. The number of peaks represent the specific phytoconstituent and the area of the peak suggests the quantity of the respective phytoconstituent present in the sample.<sup>1</sup> *Prishniparni*, botanically identified as



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*Desmodium gangeticum* (Linn.) DC. Phytoconstituents such as flavonoids, iso flavonoids, cardiac glycosides, pterocarpanes, alkaloids, steroids are seen in the root of the plant.<sup>2,3,4</sup> Previous research works suggests that the plant possess strong antioxidant and cardioprotective activities.<sup>4,5,6</sup> The oral administration of the drug in the form of *ksheerapaka* (milk decoction) has been mentioned in the Ayurvedic classics.<sup>7,8</sup> The drug can be utilized in various dosage forms. *Churna* (powder) and *ksheerapaka* (milk decoction) are two different modifications in the *Kalpana* (dosage form) of a drug. The drug when totally dried, powdered and filtered is called *churna Kalpana* (powder) and is widely utilized in therapeutics. The *churna* (powder) is a modified form of *kalka kalpana* (bolus). *Ksheerapaka* (milk decoction) is a modification of the *Kashaya Kalpana* (decoction). The HPTLC technique was employed to compare the peaks obtained for *Churna* (powder) and *ksheerapaka* (milk decoction) of dried root of *Desmodium gangeticum* (Linn.) DC. using same solvent, mobile phase and stationary phase. The present study aims at comparing the peak obtained from HPTLC chromatogram of *Churna* (powder) and *ksheerapaka* (milk decoction) of dried root of *Desmodium gangeticum* (Linn.) DC.

## MATERIALS AND METHODS

### Preparation of *churna* (powder)

*Prishniparni-Desmodium gangeticum* (Linn.) DC. was collected from Nedumoncavu village, Kollam district. The fresh root was visually inspected for foreign matter and sorted. Then it was washed with water thoroughly to remove physical impurities like soil, mud etc. Fresh root of the drug was shade dried. After proper drying it was chopped into small pieces. It was then made into fine powder and sieved through mesh with size-120.

### Preparation of *Ksheerapaka* (milk decoction)

*Ksheerapaka* was prepared as per the reference mentioned in *Sarangadhara samhitha*.<sup>9</sup> The fresh roots of *Prishniparni-Desmodium gangeticum* (Linn.) DC. were collected and dried as mentioned above. After proper drying it was chopped into small pieces. It was then made into coarse powder and sieved through mesh with size-40. The *ksheerapaka* was prepared by taking drug, milk and water in the ratio 1:8:32 respectively as mentioned in *Sarangadhara samhitha*.<sup>9</sup> For the preparation of the *ksheerapaka* initially 31.25g of the drug was added followed by addition of 250ml milk which was followed by the addition of one litre water. The mixture was then

boiled and reduced to the quantity of milk.

### Figure 1:Figure 2: Under preparation of *Ksheerapaka* (milk Decoction)

#### Procedure

Test solutions were made with 2gram of *churna* (powder) and 2ml of *ksheerapaka* (milk decoction) of dried root of *Prishniparni –Desmodium gangeticum* (Linn.) DC. extracted in 5ml methanol and 8µL was applied on the stationary phase. Stationary phase was HPTLC Silica gel 60 F 254, 4.0 x10.0 cm aluminium sheet. Mobile phase was Toluene: Ethyl acetate: Formic acid (6:3:0.1). Plates were then dried at 60°C for 5 minutes and transferred to CAMAG TLC scanner (Scanner-171019) under UV 254nm and 366 nm. Post chromatographic derivatization was done using anisaldehyde sulphuric acid 100ml and the plates were dried using oven at 120°C for 20 minutes. Densitometry was done using slit dimension of 8.00 x0.0.90 mm, Macro and the scanning speed was 20mm per seconds.

## RESULT

### A. HPTLC finger printing profile of powder of dried root of *Prishniparni [Desmodium gangeticum* (Linn.) DC.]

#### Area and peaks of Methanol extract at 254nm (Table-1, Figure -3 )

Total 9 peaks were obtained for methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 254 nm. These 9 peaks were defined with max Rf value of 0.04 with area 23389.2AU, max Rf value of 0.09 with area 10448.3 AU, max Rf value of 0.19 with area 6105.6AU, max Rf value of 0.34 with area 29924.4AU, max Rf value of 0.44 with area 13259.8 AU, max Rf value of 0.56 with area 5095.0AU, max Rf value of 0.71 with area 1757.9AU, max Rf value of 0.77 with area 819.3AU, max Rf value of 0.91 with area 4470.6 AU respectively, which are tabulated as follows.

Table No: 1 Area and peaks of methanol extract of dried root powder at 254nm Figure 3: Overview graph of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 254nm

#### Area and peaks of Methanol extract at 366 nm (Table-2, Figure -4,5,6,7,8 )

Total 10 peaks were obtained for methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. The peaks were obtained with max Rf value of 0.01 with area 721.2, max Rf value of 0.14 with area 1847.7AU, max Rf

value of 0.21 with 2135.8 AU, max Rf value of 0.34 with area 8346.0 AU, max Rf value of 0.41 with 4649.6 AU, max Rf value of 0.45 with area 6837.2AU, max Rf value 0.48 with area 4379.5 AU, max Rf value of 0.58 with area 3979.0AU, max Rf value 0.71with area 3505.1 AU, max Rf value of 0.82 with area 16729.5 respectively, which were tabulated as follows.

Table No: 2 Area and peaks of methanol extract of dried root powder at 366nm B. HPTLC finger printing profile of *ksheerapaka* of the dried root of *Prishniparni* [*Desmodium gangeticum* (Linn.) DC.]

### **B. HPTLC finger printing profile of *ksheerapaka* of dried root of *Prishniparni* [*Desmodium gangeticum* (Linn.) DC.]**

#### **Area and peaks of methanol extract at 254nm (Table-3, Figure -9)**

At 254nm, methanol extract of *ksheerapaka* of the dried root of *Desmodium gangeticum* (Linn.) DC. showed 5 peaks. The peaks were obtained with max Rf value of 0.03 with area 1020.8 AU, max Rf value of 0.33 with area 1240.3 AU, max Rf value 0.79 with area 268 AU, max Rf value 0.86 with area 553 AU, max Rf value 1.05 with area 697.2 AU respectively, which were tabulated as follows.

Table No: 3 Area and peaks of methanol extract of *ksheerapaka* of dried root at 254nm Figure 9: Overview graph of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. at 254nm.

#### **Area and peaks of methanol extract at 366nm (Table-4, Figure -10,11,12,13,14)**

Methanol extract of *ksheerapaka* of the dried root of *Desmodium gangeticum* (Linn.) DC. showed 8 peaks at 366nm and these peaks were having max Rf value of 0.06 with area 906.0 AU, max Rf value of 0.28 with area 1825.7 AU, max Rf value of 0.32 with area 976.8 AU, max Rf value of 0.33 with area 1303.3 AU, max Rf value of 0.48 with area 3964.1AU, max Rf value of 0.56 with area 521.1 AU, max Rf value of 0.89 with area 580.1AU, max Rf value of 1.06 with area 550.0AU respectively, were tabulated as follows. Table No: 4 Area and peaks of methanol extract of *ksheerapaka* of dried root at 366nm

### **DISCUSSION**

HPTLC fingerprinting profile regarding the dried root powder and *ksheerapaka* (milk decoction) of *Desmodium gangeticum* (Linn.) DC. was done in the present study. HPTLC chromatogram of the methanol extracts of *churna*

(powder) of dried root and *ksheerapaka* (milk decoction) of *Desmodium gangeticum* (Linn.) DC. at 254nm and 366nm were recorded. The Rf values of the separated compounds of each extract were noted at 254nm and 366nm. Each peak indicates presence of a specific chemical constituent. At 254nm, methanol extract of the *churna* (root powder) showed presence of maximum number of peaks (9 peaks), while the *ksheerapaka* (milk decoction) showed presence of 5 peaks. There was no presence of similar Rf in both samples and peak intensities were found to be different in both the samples. At 366 nm methanol extract of the *churna* (root powder) showed presence of maximum number of peaks (10 peaks), while the *ksheerapaka* (milk decoction) showed presence of 8 peaks. There was presence of a similar Rf value in both samples and peak intensities were found to be different in both the samples, further indicating the presence of same chemical constituent. Maximum Rf value of 0.48 in *churna* and *ksheerapaka* showed an area of 4379.5 and 3964.1 respectively. Here, the peak area is reduced in *ksheerapaka*, indicating that the concentration of the chemical constituent was reduced in it. The number of peaks and the intensities were found to be more in the *churna* (root powder) compared to *ksheerapaka* (milk decoction) at 254nm and 366nm with comparable peaks in both suggesting the presence of the same chemical constituent in different quantities. Maximum resemblance in the number of peaks and peak intensities were noted at 366nm.

### **CONCLUSION**

HPTLC profiling of *churna* (powder) and *Ksheerapaka* (milk decoction) of *Desmodium gangeticum* (Linn.) DC. was conducted and comparison of their peaks were discussed. The results suggest comparable peaks in *churna* (powder) and *ksheerapaka* (milk decoction) at 254nm and 366nm with comparable peaks in both suggesting the presence of the same chemical constituent in different quantities. Maximum resemblance in the number of peaks and peak intensities were noted at 366nm. The intensity and number of peaks were found to more in the *churna* (powder).

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**Conflict of Interest:** Nil

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Figure 1: *Churna* (powder) of dried root of *Desmodium gangeticum* (Linn.) DC.



Figure 2: *Ksheerapaka* (milk decoction) of dried root of *Desmodium gangeticum* (Linn.) DC.

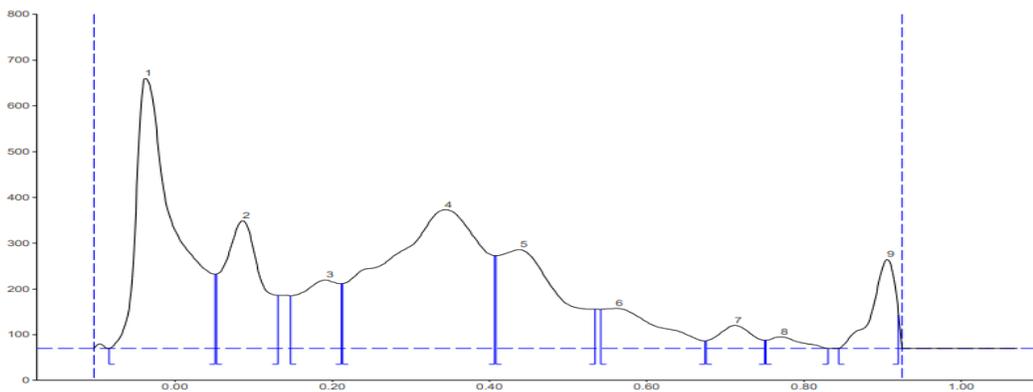


Figure 3: Overview graph of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 254nm

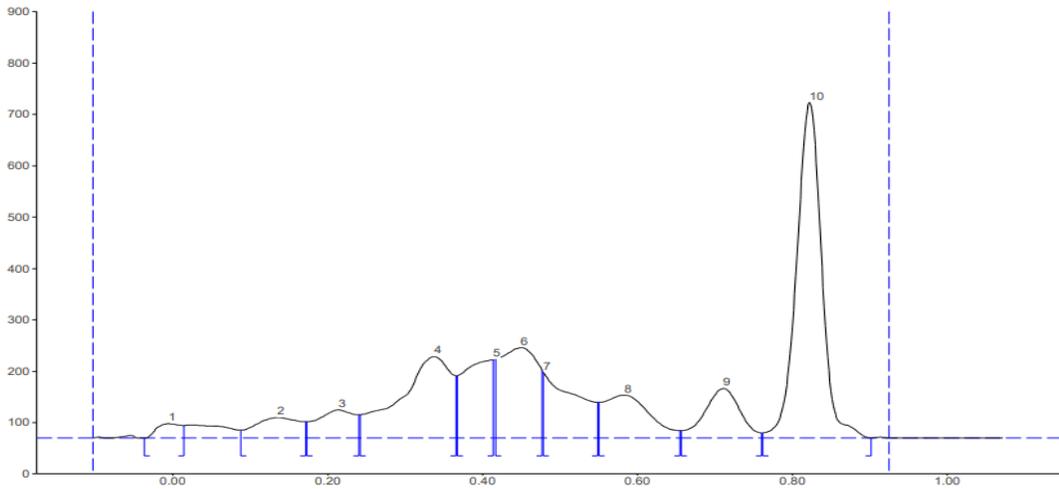


Figure 4: Overview graph of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 366nm

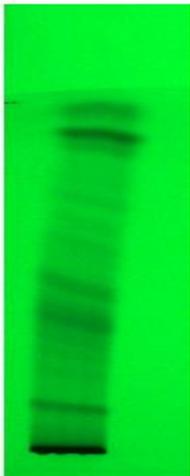


Figure 5: TLC views of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 254nm

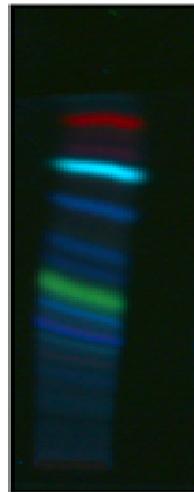
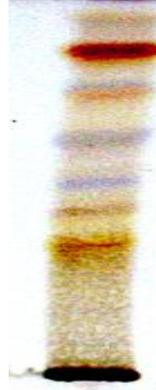


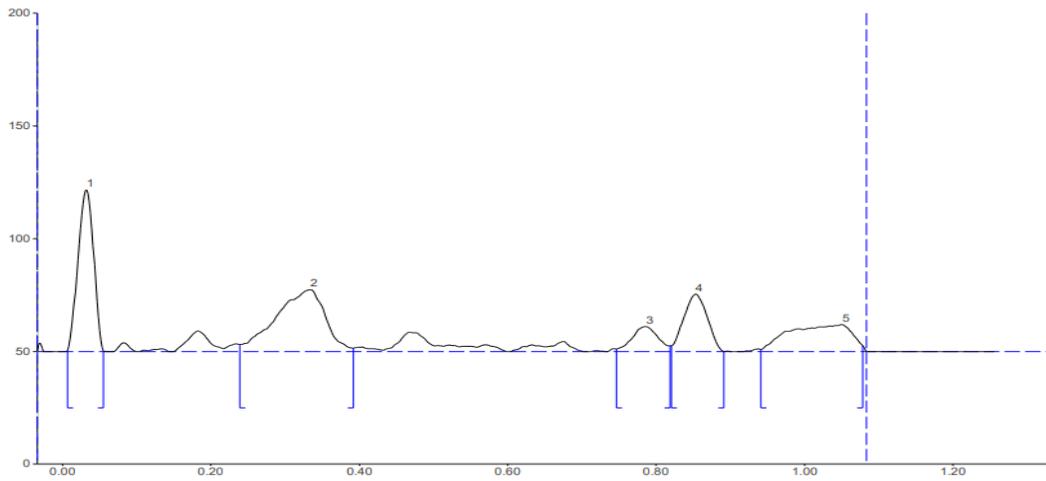
Figure 6: TLC views of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. at 366nm



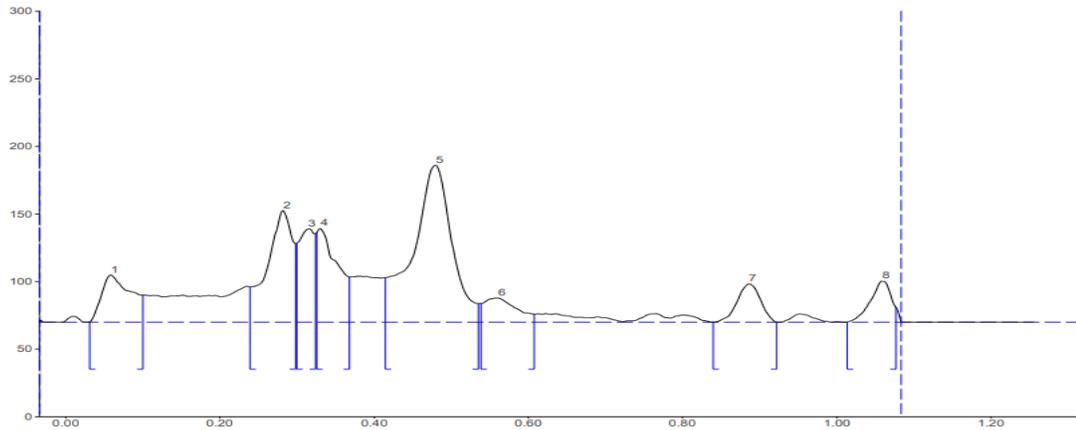
**Figure 7:** TLC views of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. after derivatization at 366nm



**Figure 8:** TLC views of methanol extract of dried root powder of *Desmodium gangeticum* (Linn.) DC. after derivatization in white light



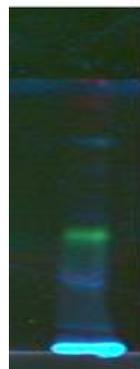
**Figure 9:** Overview graph of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. at 254nm.



**Figure 10:** Overview graph of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. at 366 nm.



**Figure 11:** TLC views of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. at 254 nm.



**Figure 12:** TLC views of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. at 366 nm.



**Figure 13:** TLC views of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. after derivatization at 366nm.



**Figure 14:** TLC views of methanol extract of *ksheerapaka* of dried root of *Desmodium gangeticum* (Linn.) DC. after derivatization in white light.

**Table No: 1 Area and peaks of methanol extract of dried root powder at 254nm**

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	-0.08	-0.04	0.05	23389.2	24.55
2	0.05	0.09	0.13	10448.3	10.97
3	0.15	0.19	0.21	6105.6	6.41
4	0.21	0.34	0.41	29924.4	31.41
5	0.41	0.44	0.54	13259.8	13.92
6	0.54	0.56	0.68	5095.0	5.35
7	0.68	0.71	0.75	1757.9	1.85
8	0.75	0.77	0.83	819.3	0.86
9	0.85	0.91	0.92	4470.6	4.69

**Table No: 2 Area and peaks of methanol extract of dried root powder at 366nm**

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	-0.04	-0.01	0.01	721.2	1.36
2	0.09	0.14	0.17	1847.7	3.48
3	0.17	0.21	0.24	2135.8	4.02
4	0.24	0.34	0.37	8346.0	15.71
5	0.37	0.41	0.41	4649.6	8.75
6	0.42	0.45	0.48	6837.2	12.87
7	0.48	0.48	0.55	4379.5	8.24
8	0.55	0.58	0.66	3979.0	7.49
9	0.66	0.71	0.76	3505.1	6.60
10	0.76	0.82	0.90	16729.5	31.49

**Table No: 3 Area and peaks of methanol extract of *ksheerapaka* of dried root at 254nm**

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	0.01	0.03	0.06	1020.8	27.01
2.	0.24	0.33	0.39	1240.3	32.82
3.	0.75	0.79	0.82	268.0	7.09
4.	0.82	0.86	0.89	553.0	14.63
5.	0.94	1.05	1.08	697.2	18.45

**Table No: 4 Area and peaks of methanol extract of *ksheerapaka* of dried root at 366nm**

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	0.03	0.06	0.10	906.0	8.53
2.	0.24	0.28	0.30	1825.7	17.18
3.	0.30	0.32	0.32	976.8	9.19
4.	0.33	0.33	0.37	1303.3	12.26
5.	0.42	0.48	0.54	3964.1	37.30
6.	0.54	0.56	0.61	521.1	4.90
7.	0.84	0.89	0.92	580.1	5.46
8.	1.02	1.06	1.08	550.0	5.18