

## COMPARATIVE PHYTOCHEMICAL STUDY OF SHALAPARNI AND SHALAPARNI KSHEERAPAKA

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### ABSTRACT

Ayurved classics are a rich compendium of several drugs and their combinations for treatment in various diseases. Dosage forms play a vital role in effective efficacy of the drug. Shalaparni a important formulation of Dashamula is stated to be used in the form of ksheerapaka in hridayagata vata. Ksheerapaka is a unique dosage form of ayurved. Ksheerapaka has benefits of they are good for heart and are palatable. So a study was carried to understand differences phytochemically, this revealed presence of flavonoids, alkaloids, glycosides in Shalaparni and Shalaparni Ksheerpaka.

### INTRODUCTION

Several drugs and thereby formulations have been mentioned in Ayurveda. Drugs are used in combination or special dosage forms have been suggested for optimum result. *Shalaparni* is one of the drugs stated in *Dashamula*, a popular and important formulation with multitude uses. The drug is easily available, economical and safe with no adverse effects reported so far. *Shalaparni* (*Desmodium gangeticum*(L.) DC.) *Ksheerpaka* has been advocated in the treatment of *Hridayagatavata*.<sup>[1]</sup> Ksheerapaka is a unique formulation stated in Ayurveda. Few drugs have been stated to be used in the form of Ksheerpaka. A popular example being Arjuna ksheerapaka. A phytochemical study was therefore conducted to understand the changes in the chemical constituent of there drug with a different processing ie ksheerapaka.

## MATERIAL AND METHODS

### Drug Collection

*Shalaparni* (*Desmodium gangeticum*(L).DC). Roots were collected from natural habitat through a Botanist Mr. Chellabery, Retd. Scientist Regional Research Institute Siddha Medicine from Tirumelveli, Tamilnadu during GrishmaRitu. Classical texts advocate the collection of roots in Grishma Ritu.<sup>[2]</sup> Only Lateral roots were collected for conservation purposes in accordance with Good Agricultural and Collection Practices, WHO guidelines 2003.<sup>[3]</sup>

### Authentication

The test drug was authenticated by Botanist, Voucher specimen no. (#: **rbj p 0741118**) and deposited for reference.

Guidelines for standardization of drug is given by *AcharyaCharak* in *Vimansthan* 8/87, which is relevant with monogram given by W.H.O. Study drug was compared with the standards mentioned in the *Ayurvedic* text.<sup>[4]</sup>

Following analytical study of root of *Shalaparni* [*Desmodium gangeticum* (Linn.) DC.] and its *Ksheerpaka* were done.

1. Physicochemical study
2. Phytochemical study
3. TLC
4. HPTLC

### Preparation of Ksheerpak<sup>[5]</sup>

Cow's milk was added with thirtytwo times of water, eight times of milk and one part of *Shalaparni* root Churna(1:8:32)

The mixture was boiled on low flame i.e mandagni until only milk remains i.e. 50ml by letting water to be evaporated. This is called as *Shalaparni siddha Ksheera*. This is filtered and administered to the patients.

*Shalaparni* root powder: 10 gm.

Cow Milk: 80 ml.

Water: 320ml.

For preparation of *Ksheerapaka*

Dose of ksheerapaka -50ml<sup>[6]</sup>

**(Therapeutic usage)**

*Shalaparni* in form of *Ksheerpaka* is used in Hridayagata vata<sup>[7]</sup>

**Standardization According to API Guidelines**

**1. Identification & Authentication of Samples**

Based on the methods laid by API, physicochemical and phytochemical studies were carried.

**2. Organoleptic Characters**

Organoleptic evaluation of drug i.e. evaluation of drug based on sensory profile by Colour, Odour, Size, Shape, Taste were performed.

**3. Macroscopic Examination**

Roots of *Shalaparni* were observed for size, shape, surface etc.

**4. Microscopic Study**

**Microscopic characters of Root**

Root sample were cut into very thin slices with the help of blade and were dipped in water for 6-12 hours' time to soften them. After that staining was done with safranin. Post staining, the section was mounted on slide and observed.

**PHYSICOCHEMICAL ANALYSIS**

a. Determination of Moisture Content (Loss on Drying)

b. Determination of Ash Values

1. Determination of Total Ash

2. Determination of Acid Insoluble Ash

c. Determination of Solvent Extractive Values

1. Determination of Alcohol Soluble Extractive

2. Determination of Water-Soluble Extractive Value

d. Determination of pH Values

**Physico-Chemical Analysis<sup>[8]</sup>**

Loss on drying (Moisture content)

**DETERMINATION OF FOREIGN MATTER<sup>[9]</sup>**

The drug used was studied for the presence of moulds, insects, animal faecal matter, fibres and other contaminations such as soil, stones and extraneous material which is considered as foreign matter and its percentage was calculated.

**Procedure**

- 100 grams of both samples of *Shalaparni* moola were taken
- Samples was spread on a white surface uniformly without overlapping.
- The samples were inspected with naked eye and also with 5x lens.
- The foreign organic matter was separated (mentioned above) manually.
- After complete separation, the matter was weighed and the percentage was determined w/w present in the sample.

**Total Ash value estimation:** 2 grams of the air dried drug was weighed accurately in a silica dish and incinerated at a temperature not exceeding 600°C for 3 hours until free from carbon, cool and weigh. Then the percentage of ash with reference to air-dried drug was calculated.

**Acid Insoluble Ash**

The ash obtained by the above method mentioned was boiled . 25 ml of dilute hydrochloric acid was added. The acid insoluble ash was collected in a pre weighed crucible along with the ash less filter paper kept in muffle furnace for an hour at around 450°C ± 5°C. The percentage of acid insoluble ash with reference to the air dried drug was calculated

$$\text{Acid insoluble ash} = \frac{\text{Weight of ash} \times 100}{\text{Original sample weight}}$$

**Determination of Water Soluble Extractive**

5grams of the powdered drug was taken in a weighing bottle and transferred to a dry 250ml conical flask. 100ml-graduated flask was filled to the delivery mark with chloroform water (1.25 ml chloroform + 500ml distilled water). The weighing bottle was washed washings were poured together with the remainder of the solvent into the conical flask.

The flask was corked and set aside for 24 hours shaking frequently, then filtered in to a 50ml cylinder. When sufficient filtrate was collected, 25ml of the filtrate was transferred to a Weighed thin porcelain dish as used for the ash value determination. It was then Evaporated

to dryness on a water bath and complete the drying in an oven in a 100°C, then cooled. The percentage of extractive with reference to the air-dried drug was calculated.

#### **Determination of Alcohol Soluble Extractive**

- 5g of the powdered drug was taken in a weighing bottle and transferred to a dry 250 ml conical flask. 100ml-graduated flask was filled with ethanol. Weighing bottle was washed out and poured together with the remainder of the solvent into the conical flask.
- The flask was corked and set aside for 24 hours shaking frequently. Then filtered in to a 50ml cylinder. It was then evaporated to dryness on a water bath and then dried in an oven at 100°C, then cooled. The percentage of extractive with reference to the air-dried drug was calculated.

#### **pH Value<sup>[10]</sup>**

pH is useful for ensuring identity, stability and detect that the drug is free from the contamination of water soluble adulterants which are acidic or alkaline in nature as each and every drug possesses its own acidity or alkalinity measured in pH range at a specific concentration.

Determination of pH- 1 gm of powdered drug was taken in 100ml of distilled water and extracted for 24 hours. The extract was taken for measuring the pH value prior to the various procedures. At first the pH meter electrode was soaked in the buffer of pH 7 for eight hours at room temperature and after that kept on standby mode so that it could be readily used for measurement. The reference electrode was filled with set solution and air bubbles were taken out. The electrode was also tested for buffer of pH 4 to check the accuracy. The prepared extract was taken and the pH was measured.

#### **Preliminary Phytochemical Screening**

- Aqueous, alcoholic extracts of the test drug were further subjected for qualitative preliminary photochemical screening.

#### **Test for Alkaloid**

1ml of extract plus 5ml of dilute HCL Keep in water bath at 60°C for 15min add 1ml of wagners reagent Reddish brown precipitate indicates presence of Alkaloids.

#### **Test for Flavonoid**

1ml extract plus 1ml of NaOH yellow colour turns colourless after adding dil. H<sub>2</sub>SO<sub>4</sub>. This indicates presence of flavonoids.

### Test for Glycosides

1ml of extra plus 1ml of glacial acetic plus 3% FeCl<sub>3</sub>. Add conc. H<sub>2</sub>SO<sub>4</sub> from side Brown ring formation indicates presence of Glycosides.

### Thin Layer Chromatography

T.L.C is one of the most widely used techniques for rapid identification of drugs and its formulations. It is equally applicable to the drugs as raw material state and pure state.

### Chromatographic Conditions

Shalparni root, and Shalparni kheerpaka were subjected for thin layer chromatography as follows,

**Preparation of Tlc:** Pre coated Silica Plate was used.

### Sample Preparation

1. 1gm of Sample was weighed.
2. 10ml of ethanol was added.
3. Kept in rotary for 24hr.
4. The sample was filtered using Whatman filter Paper no 41.
5. The excess solvent was evaporated.
6. The obtained extract was reconstituted in 1ml of ethanol.

### Chromatographic Conditions

Stationary phase – Aluminium coated silica plates 60 F(254).

Saturation Time – 20 min.

Sample application volume – 5ml.

Mobile phase for flavonoids – Ethyl acetate: water: formic acid: glacial acetic acid (10:0.5:0.5:0.5) (v/v/v/v).

Mobile phase for glycosides – ethyl acetate: water (10:1.5:0.5) (v/v/v).

Mobile phase for alkaloids – toluene: ethylacetate: water (7:2:1) (v/v/v).

This solvent system holds good and clear appearances of bands (spots) were seen.

### Interpretation for TLC

TLC was done of the whole root and the extract. Samples were subjected to various mobile phase depending on the compound of interest.

**HPTLC:** Was performed for both samples i.e. *Shalaparni* and *ksheerapaka*.

### OBSERVATION

#### Observations and Results

#### Pharmacognostic Study

**Table No. 1: Organoleptic characters of Root of *Shalaparni*(*Desmodium gangeticum*L. DC).**

S.no	Organoleptic Characters	<i>Shalaparni root</i>	<i>Shalparni Ksheerpaka</i>
1	Shape	Long ,thin	.....
2	Size	Lateral roots 15-30cm long and 0.1-0.8cm thick	.....
3	Surface /Texture	Rough, powder fine	Appearance-Hazy liquid
4	Colour	Yellow	Light Creamish brown
5	Odour	Faint	Characteristic
6	Taste	Tasteless, Classics-Tikta-Madhura	Characteristic/Ayurved. Tikta-Madhur

#### Macroscopic Characteristics of *Shalparni* Root

The roots are uniformly cylindrical with lateral roots lenticles nodules. Lateral roots size 15-30cm long and 0.1-0.8cm thick, Outer surface smooth bearing a number of transverse, light brown lenticels.

#### Microscopic Characteristics of T.S of *Desmodium gangeticum*(*Shalaparni*) Root

Internally the outermost layer is thin layered cork with elongated rectangular cells, next is cortex thin walled ovate shaped parenchyma cells and sclerechymatous fibers, phloem polygonal to rectangular cells around central wood which has vessels, parenchyma, fibers. The xylem is diffused porous.

**Table no. 2: Physicochemical Analysis of *Shalparni* Root (*Desmodium gangeticum* L.DC)**

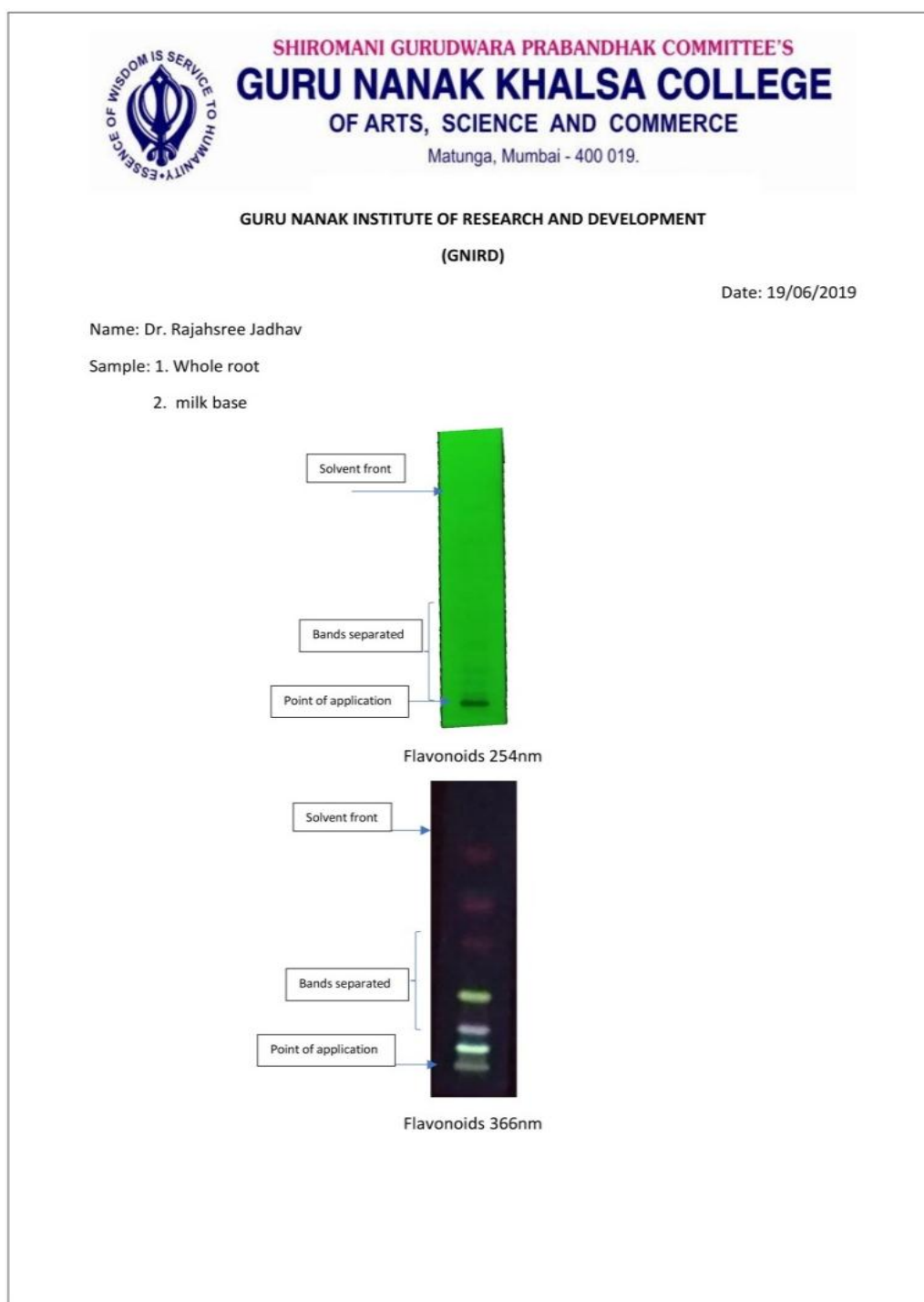
Particulars	<i>Shalparni</i> Root	<i>Shalaparni</i> Ksheerapaka	API Standard
Total Ash value (% w/w)	3.58	-	Not more than 6 %
Acid insoluble ash (% w/w)	0.89	-	Not more than 2 %
Moisture content (% w/w)	3.8	-	Not more than 5 %
Alcohol soluble	5.87	-	Not less than 1 %

extractive (% w/w)			
Water soluble extractive (% w/w)	7.35	-	Not less than 6 %
PH	-	6.6	
Total Solid		8.32 %	

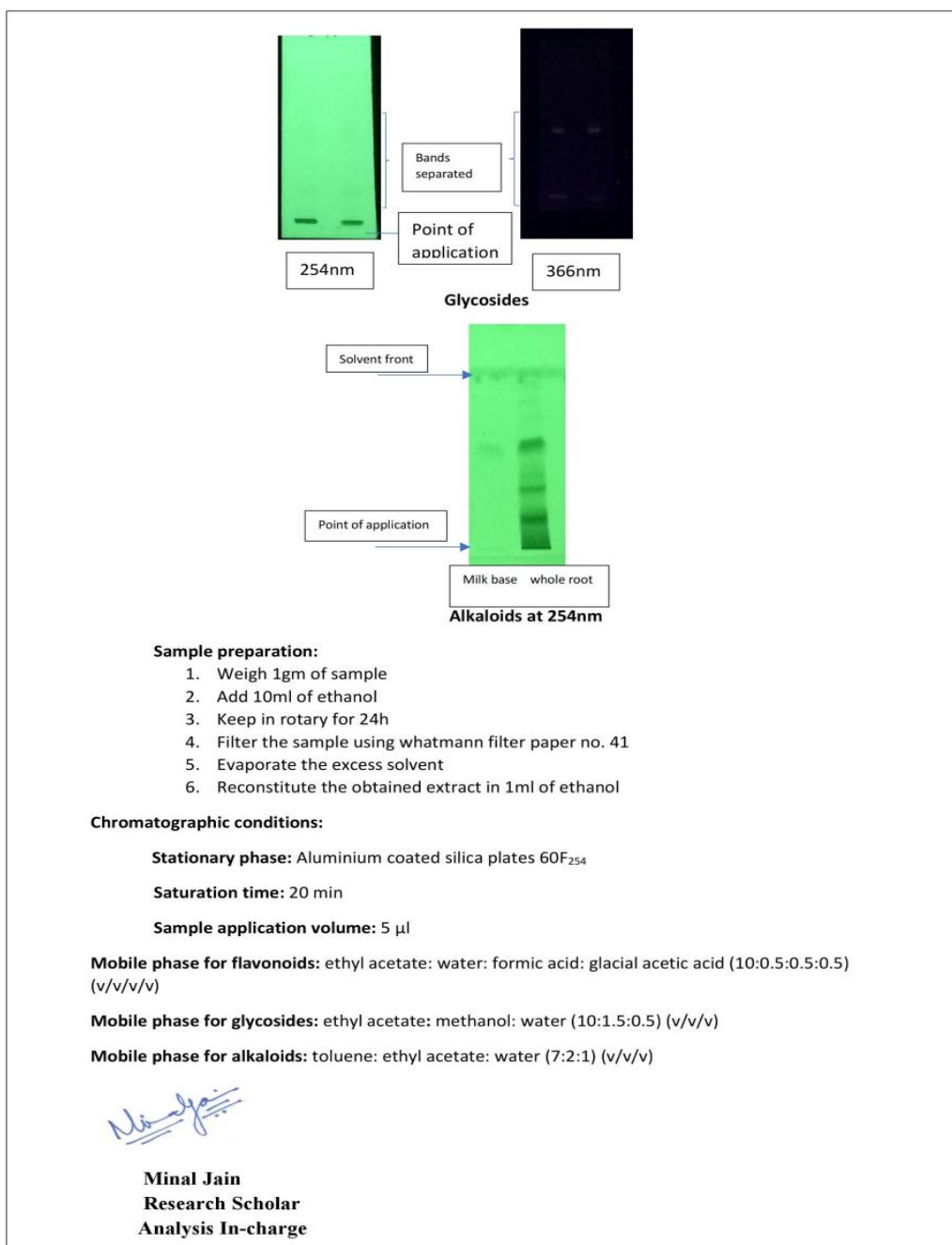
**Table no. 3: Phytochemical Analysis of Test Samples.**

<b>Chemical constituents</b>	<b>Observation</b>	<b><i>Shalaparni</i> Root</b>	<b><i>Shalaparni</i> <i>Ksheerpaka</i></b>
Alkaloids	Reddish brown precipitate observed	Present	Present
Glycosides	Brown ring formed	Present	Present
Flavonoids	Colourless	present	present






**Image No. 1: TLC findings of Shalaparni root & Shalaparni Ksheerpaka.**



**Image No.2: TLC findings of *Shalaparni* root & *Ksheerpaka*.**

### Interpretation for TLC

TLC was done of the whole root and the extract. Samples were subjected to various mobile phase depending on the compound of interest. Flavonoids were found to be present in the given sample. More number of flavonoid components were observed when plate was scanned at 366nm. Glycosides were also observed at 366nm. Alkaloids showed a better range of bands when scanned at 254nm. Thus, it can be concluded that the sample as well as the shirpak both contained the flavonoids, glycosides and alkaloids. The same was confirmed and quantified using HPTLC. Similar results were obtained.



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Name: Dr. Rajashree Jadhav  
 Date: 19/06/2019  
 No. of sample: 02  
 Test: Phytochemical screening

1. Alkaloid
2. Flavonoid
3. Glycoside

Sample [preparation;  
 1gm of sample in 10ml of ethanol.  
 Filter using whatmann filter paper no. 41.


Test for alkaloids:  
 1ml of extract + 5ml of dilute HCl. Keep in water bath at 60°C for 15 min. add 1ml of wagners reagent. Reddish brown precipitate indicates presence of alkaloids.

Test for flavonoids:  
 1ml of extract + 1ml of NaOH. Yellow color turns colorless after adding dil. H<sub>2</sub>SO<sub>4</sub>. This indicates presence of flavonoid.

Test for glycosides:  
 1ml of extract + 1ml of glacial acetic acid + 3% FeCl<sub>3</sub>. Add conc. H<sub>2</sub>SO<sub>4</sub> from side. Brown ring formation indicates presence of glycosides.

Result:

Test	Alkaloid	Flavonoid	Glycoside
Whole root	+	+	+
Milk base	+	+	+



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**Image No.3: Phyto-chemical Screening of *Shalaparni* root and *Shalaparni* Kshreepaka.**

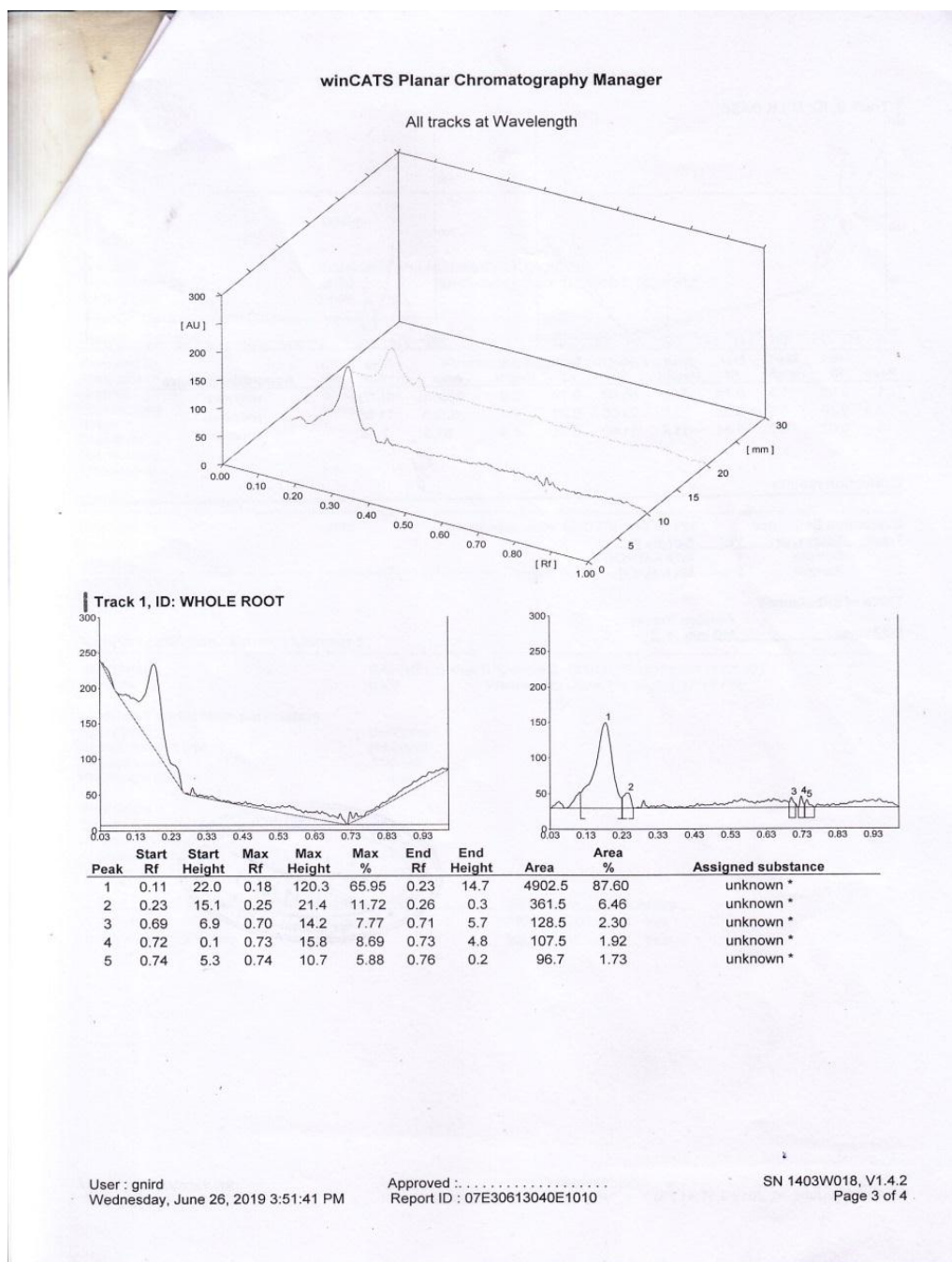


Image No. 4: HPTLC of Shalaprni root &amp; Ksheerpaka.

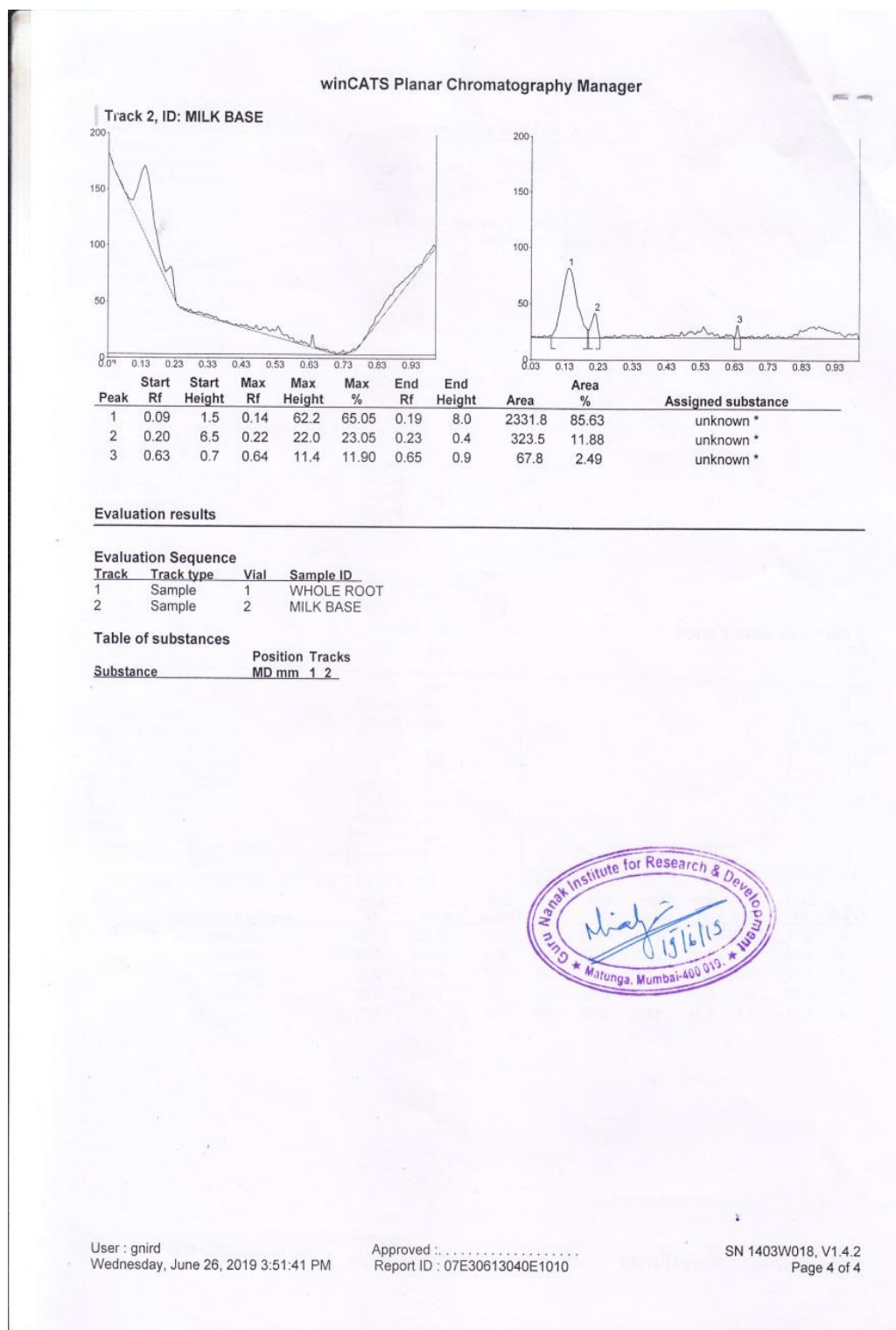


Image No. 5: HPTLC of Shalaprni root &amp; Ksheerpaka.

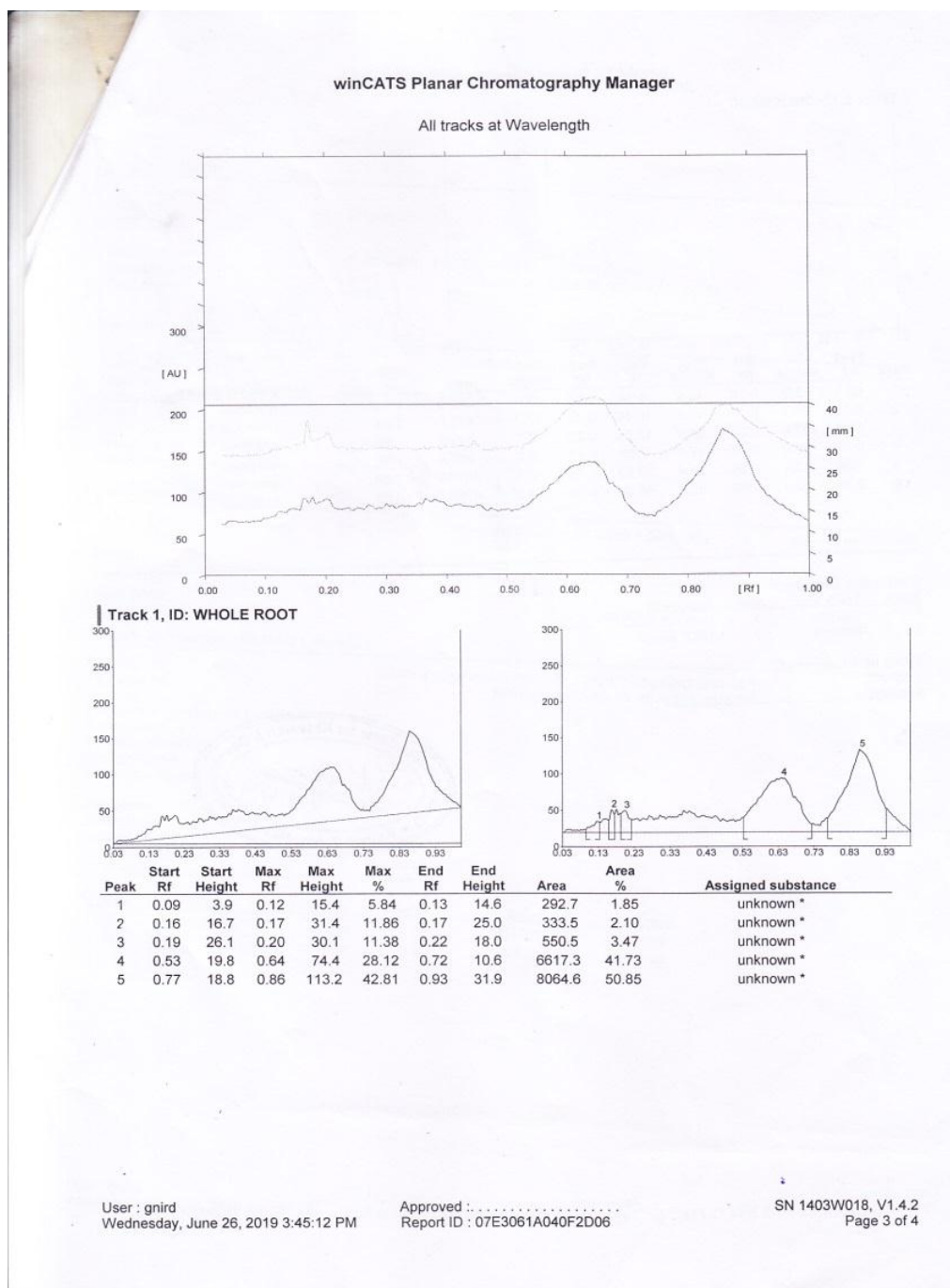


Image No. 6: HPTLC of Shalaprni root &amp; Ksheerpaka



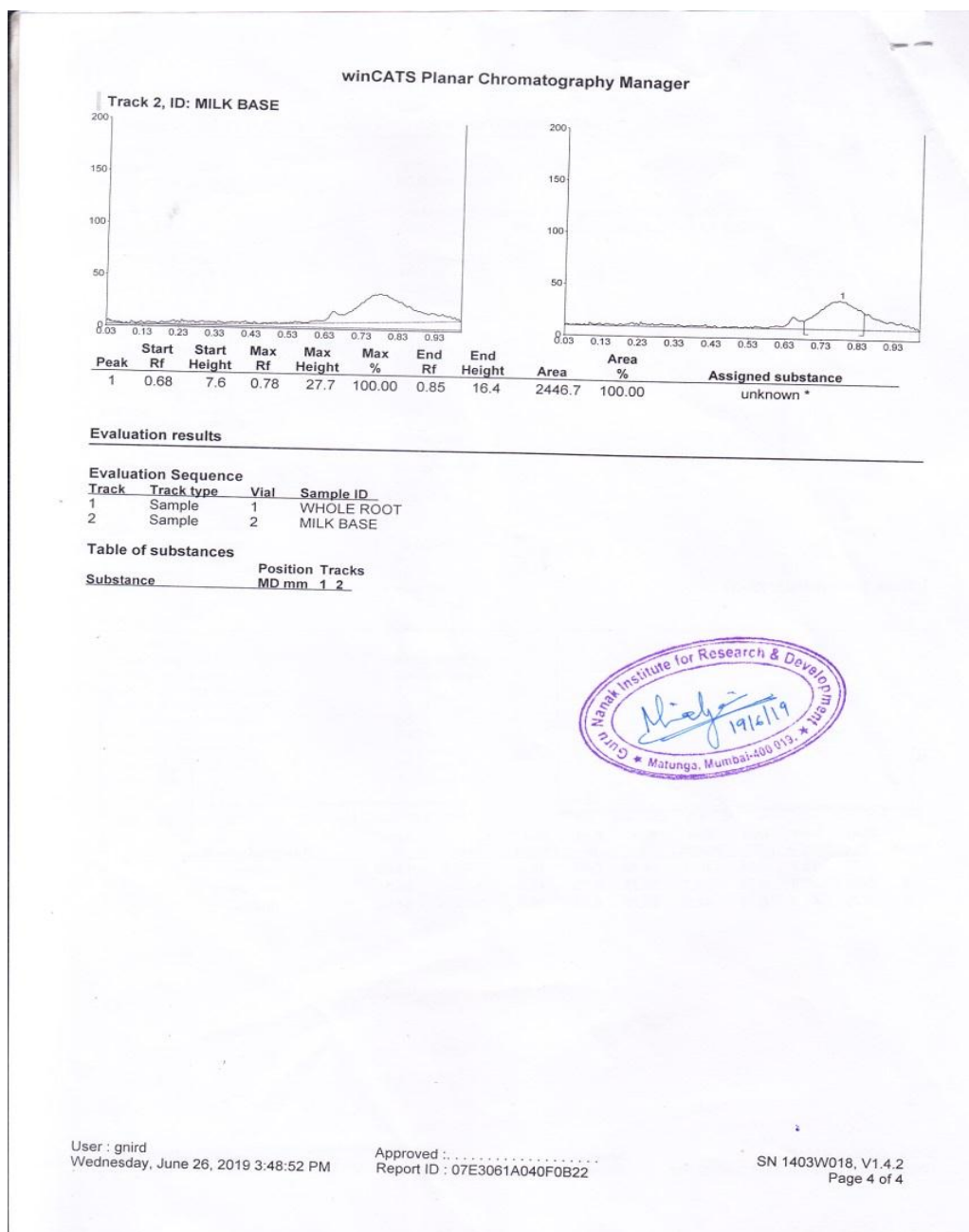


Image No.7: HPTLC of Shalaprni root &amp; Ksheerpaka.

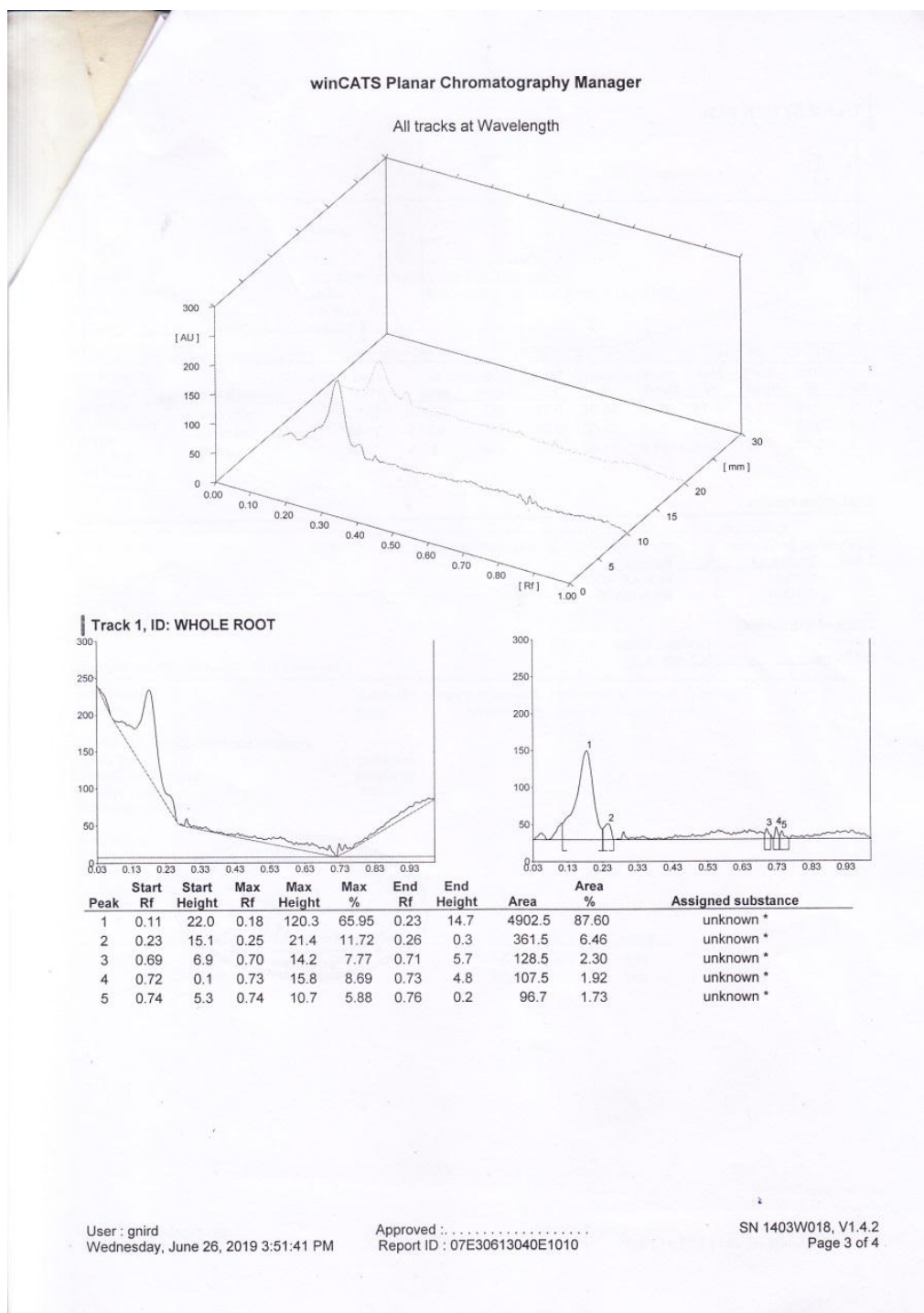
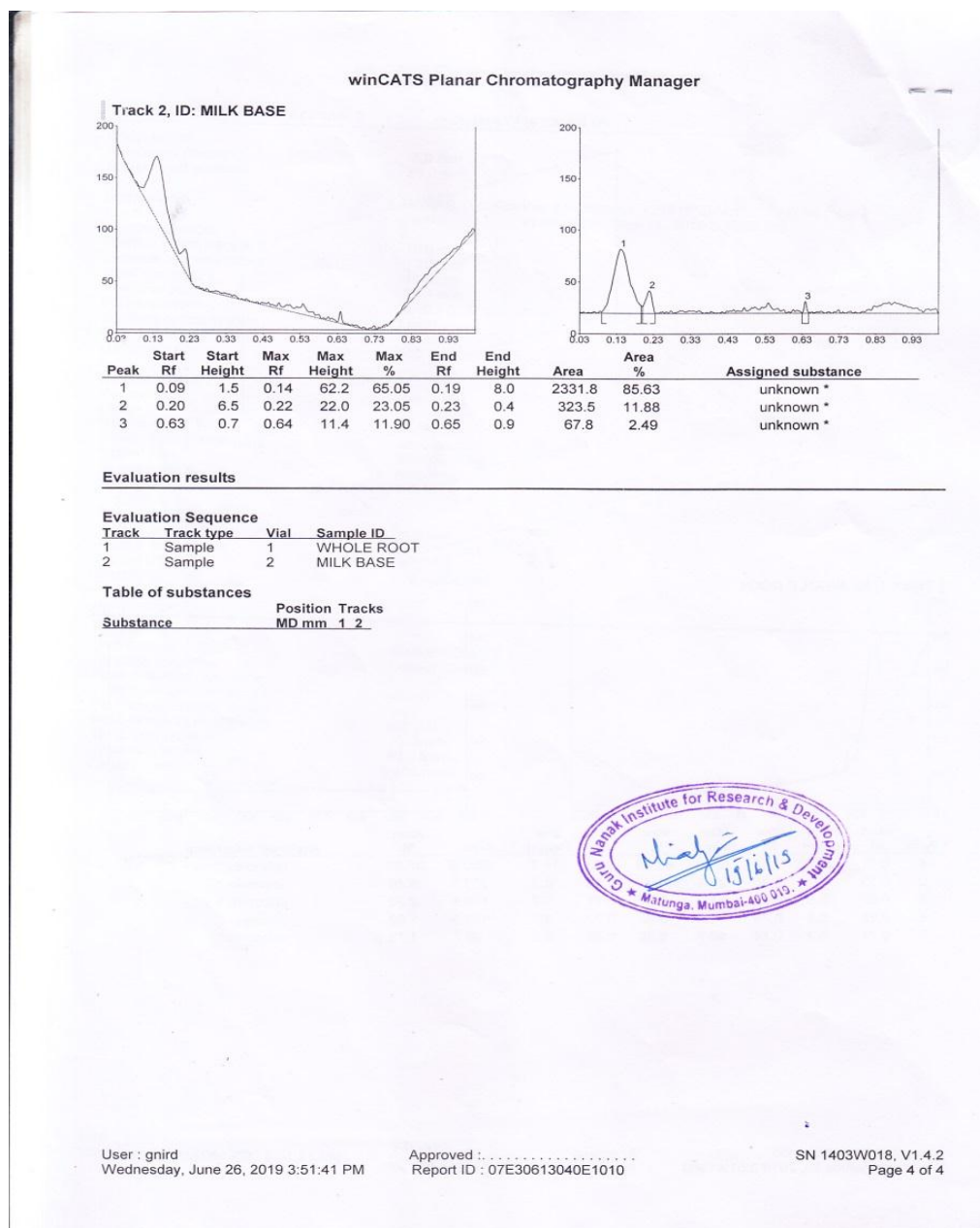


Image No. 8: HPTLC of Shalaparni root &amp; Ksheerpaka.





**Image No. 9: HPTLC of Shalaparni root & Ksheerpaka.**

## DISCUSSION

In the chapter of Vatavyadhi Acharya *Charaka* has advised *Shalaparniksheerapaka* for the treatment of Hridayagatavata.<sup>[7]</sup> Several species of *Shalaparni* are available of which predominant are *Desmodium gangeticum* L.DC, *Desmodium repandum* L.DC, *Desmodium lexiciflorum* L.DC, *Desmodium diffusum* L.DC, *Desmodium triflorum* L.DC. Of these *Desmodium gangeticum* L.DC is the accepted species in API Part 1 Vol. 3. Therefore *Desmodium gangeticum* L.DC was chosen for the study.<sup>[6]</sup>

*Shalaparni* Root – Taproot, dried roots were uniformly cylindrical with lateral roots lenticels and nodules CORRESPONDED TO THE STANDARD accepted SAMPLE

Organoleptic characters-also match the description in the classics where *Shalaparni* is stated to have madhurarasa, Tikta.<sup>[11]</sup>

### Physicochemical study (Table No.2)

- Moisture content is important to understand the preservation of the drug. If the percentage is higher than normal limits it will lead to easy infestation with degradation of metabolites and deterioration of the quality of drug. Moisture content was 3.8% within limits. (Not more than 5% -API) suggesting that the sample is well preserved.
- Total Ash Value was 3.58% (Not more than 6 %-API) within normal limit. Ash value denotes the presence of inorganic matter and salt materials in the sample which was within normal limit indicating no adulteration of any other plant parts or stones in drugs.
- Acid Insoluble Ash Value denotes percentage of silicacious matter and mud. This was 0.89% (Not more than 2 %-API) in the test sample within API standards which suggests there was no adulteration of any earthen material or mud etc. in drugs. Poor collection, poor storage conditions or poor preservation methods may result in higher Acid insoluble content.
- Water Soluble Extractive Value was found to be 7.35% (Not less than 6 %-API) which suggests the presence of water soluble constituents such as carboxylic acids, water soluble vitamins, sugar and amino acids etc.
- Alcohol Soluble Extractive Value 5.87% (Not less than 1% -API) i.e. percentage of Active ingredients of drugs soluble in alcohol like Phenols, anthraquinones, Alkaloids, Glycoside, Flavonoids, Steroids and triterpenoids.

Both water soluble and alcohol soluble ingredients comply with API standards. Extractive values indicate the presence and solubility of active metabolites i.e. phytochemical constituents in respective solvent.<sup>[12]</sup>

PH- PH of *Shalaparni* ksheerapaka was found to be 6, while that of *Shalaparni* root is 6 as mentioned in a study. The pH of the drug did not alter after boiling in the media i.e. Milk which is used in the current study.

Thereby physicochemical parameters fall under API norms suggesting that the plant material collected is *Desmodium gangeticum* ie *Shalaparni*.

### Primary Phytochemical analysis of the Root

*Shalaparni* and *Shalaparni* Ksheerapaka were both analysed for the phytochemicals and were found positive for Alkaloids, Glycosides and Flavonoids in both the samples.

### TLC and HPTLC Findings

The analysis revealed that both the samples have flavonoids, Glycosides and Alkaloids as bioconstituents. More number of flavonoid components were observed when plate was scanned at 366nm. Glycosides were also observed at 366nm. Alkaloids showed a better range of bands when scanned at 254nm. Flavonoids were found to be present in the given sample. Thus, it can be concluded that the sample as well as the Ksheerpak both contained flavonoids, glycosides and alkaloids. The quality of phytochemicals in root does not get degraded or diluted when boiled in milk. The prepared Ksheerpak thus can be consumed as the nutritional quality remains the sample. The same was confirmed and quantified using HPTLC. Similar results were obtained. According to literature the important bio-active constituents isolated from *Shalaparni* are flavonoids, alkaloids and pterocarpanes. Among these flavonoids and alkaloids were detected in the test samples. Flavonoids are important as they are used as the reference standard. Flavonoids have a beneficial effect on hypertension as is reported by earlier studies.

### CONCLUSION

*Shalaparni* ksheerapaka is reported to be beneficial in *hridaygata vata* as flavonoids acts as antioxidant.

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