

## HPTLC FINGER PRINTING ANALYSIS OF AQUEOUS & ETHANOLIC LEAF EXTRACT THE PLANT SHIMSHAPA (*Dalbergia sissoo Roxb*)

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### ABSTRACT

Medicinal plants have been in use since centuries as a remedy for human diseases, because they contain components of therapeutic value. *Shimshapa (Dalbergia sissoo Roxb)* is known as Indian rosewood. It is useful in many conditions like *Jwara, Netra roga, Atisara, Chardi, Sopha, Vrana, Krimi, Kushta, Basthivruk* etc. HPTLC is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs. This article emphasizes on HPTLC analysis of Aqueous & ethanolic leaf extract of Shimshapa (*Dalbergia Sissoo Roxb*).

**KEYWORDS:** Shimshapa, TLC, HPTLC, Pthalic acid.

### INTRODUCTION

*Shimshapa (Dalbergia sissoo Roxb)* is known as Indian rosewood<sup>1</sup>. It is useful in many conditions like *Jwara, Netra roga, Atisara*<sup>2</sup>, *Chardi, Sopha*<sup>3</sup>, *Vrana, Krimi, Kushta, Basthivruk*<sup>4</sup> etc. Different parts of *Shimshapa (Dalbergia sissoo Roxb)* are used in indifferent formulations for the treatment in many diseases like *patra swarasa* used in eye disease, heart wood is used in fever, oil extraction is used in cutaneous infection, mucilage of the leaves used as application in excoriation. In acute stage of gonorrhoea leaf decoction is used<sup>5</sup>. In India and Nepal rural people use *Shimshapa* leaves to treat animals suffering from non-specific diarrhoea. Herbal preparation of *Shimshapa* with cow urine can be used as a potent antiseptic preparation for prevention and treatment of chronic bacterial infection<sup>6</sup>.

Standardization of herbal drugs is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility<sup>7</sup>.

HPTLC is one of the sophisticated instrumental techniques for qualitative and quantitative analysis of the herbs and herbal drugs. It is a modern analytical separation method with extensive versatility. It is used in assaying radiochemical purity of radiopharmaceuticals, the determination of pigments that a plant contains, identifying the compounds present in a given substance<sup>8</sup>.

The present study is aimed to detect presence of Pthalic acid in the Aqueous & ethanolic leaf extract of *Shimshapa*

(Dalbergia Sissoo Roxb). by HPTLC finger printing method.

### MATERIALS AND METHODS:

The aqueous and Ethanolic leaf extract of *Shimshapa* was done in H.S.K College of Pharmacy, Bagalkot. HPTLC analysis was done in Son biologicals, 1<sup>st</sup> floor, KR road, Shastrinagar, BSK 2<sup>nd</sup> stage, Bangalore.

### MATERIALS:

→ Chloroform (Manufactured by Thomas baker, Date of expiry: September 2023.)

→ Ethanol (Manufactured by RANKEM.)

→ TLC plates- TLC Silica gel 60 F254 (10 cm× 10 cm; 0.25 mm layer thickness; Merck).

→ HPTLC (CAMAG, Switzerland).

→ Test sample

### Preparation of standard

Accurately weighed 10mg of standard Phthalic acid was taken and transfer to 20 ml volumetric flask and 20 ml of ethanol was added. Sonicate the solution for 5 minutes.

### Preparation of sample:

Accurately weighed 10 mg of Aq. & Ethanolic leaf extract of *Shimshapa* was taken and transfer to 10 ml volumetric flasks, to this 10 ml of respective solvents was added. These solutions were used for T.L.C study.

### METHODOLOGY:

→ High-Performance Thin-Layer Chromatography was performed on silica

### OBSERVATIONS & RESULTS:

Sample	Rf value	Area	Dilution µg/ml	Weight in mg
Phthalic acid	0.64	6332	1000	10
Aqueous leaf extract of Shimshapa	0.64	2112.5	10000	100
Ethanolic leaf extract of Shimshapa	0.64	2613.1	10000	100

Calculation of R<sub>f</sub> value for Aq. Leaf extract of *Shimshapa*:

gel 60 F254 (10 cm× 10 cm; 0.25 mm layer thickness; Merck).

→ Aqueous and Ethanolic leaf extract of *Shimshapa* were prepared in 10ml volumetric flask by taking 250mg of the extract and diluting with respective solvent (the concentration was 25mg/ml) and filtered through a 0.45 micron syringe filter.

→ From these filetrate 4, 6, 8, 12mg/ml concentrations were subjected to HPTLC (CAMAG, Switzerland) analysis.

→ Both extracts and marker compound Phthalic acid were spotted on a silica gel 60F254 (Merck, Darmstadt, Germany) TLC plate.

→ The plate was air dried and then the solvent system was developed by using Chloroform: Methanol: Formic acid (6:4:0.1 v/v) as mobile phase in a CAMAG- twin-trough glass chamber previously saturated with mobile phase vapor for 20 min.

→ After development of spots plate was dried at 65°C for 2 min and then it was scanned using camag Scanner 3 (CAMAG, Switzerland) at 254 and 365 nm using WinCATS 4 software.

→ Refractive values were calculated.

Calculation:

$$R_f = D_s / D_f$$

Where, D<sub>s</sub> = distance traveled by the solution

D<sub>f</sub> = distance travelled by the solvent.

$$R_f = \frac{\text{Sample area}}{\text{Std area}} \times \frac{\text{Std wt}}{\text{Spl wt}} \times \frac{\text{Spl dil}}{\text{Std dil}} \times \% \text{ Purity of Std}$$

2112.5/6332x10/100x 10000/1000x99 =  
33.26 µg/ml.

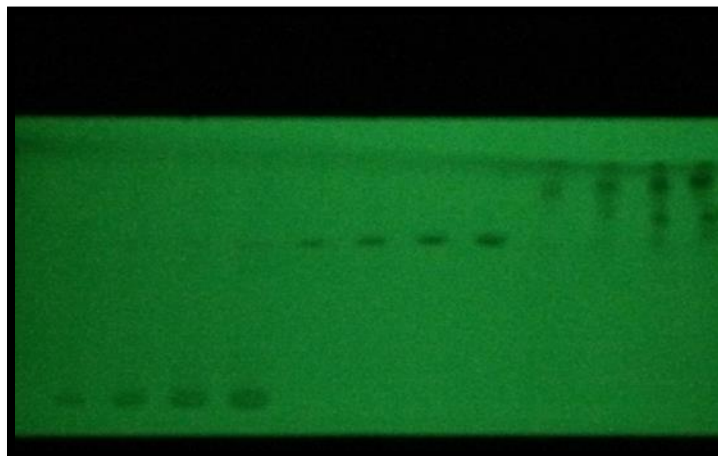
R<sub>f</sub> = 33.26 µg/ml.

**Calculation of R<sub>f</sub> value for Ethanolic Leaf extract of Shimshapa:**

R<sub>f</sub> = Sample area/Std area X Std wt/Spl wt X  
Spl dil/Std dil X %Purity of std

2613.1/6332x10/100x 10000/1000x99 =  
40.84 µg/ml.

R<sub>f</sub> = 40.84 µg/ml.



DSaq DSaq DSaq DSaq PA PA PA PA DSE DSE DSE DSE

2 4 8 12 2 4 8 12 2 4 8 12

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.69 Rf	8.0 AU	0.71 Rf	34.7 AU	00.00 %	0.76 Rf	3.2 AU	1013.8 AU	00.00 %	unknown *
2	1	0.65 Rf	8.6 AU	0.68 Rf	02.4 AU	00.00 %	0.70 Rf	5.7 AU	1352.3 AU	00.00 %	unknown *
3	1	0.64 Rf	3.8 AU	0.68 Rf	27.9 AU	00.00 %	0.73 Rf	2.2 AU	2112.5 AU	00.00 %	unknown *
4	1	0.54 Rf	9.2 AU	0.60 Rf	18.4 AU	9.72 %	0.61 Rf	3.2 AU	816.7 AU	19.01 %	unknown *
4	2	0.62 Rf	3.9 AU	0.67 Rf	54.4 AU	81.51 %	0.71 Rf	5.8 AU	2890.4 AU	67.28 %	unknown *
4	3	0.75 Rf	0.8 AU	0.79 Rf	16.6 AU	8.77 %	0.83 Rf	0.2 AU	588.9 AU	13.71 %	unknown *
5	1	0.64 Rf	5.6 AU	0.67 Rf	84.1 AU	87.19 %	0.71 Rf	0.9 AU	6332.0 AU	84.97 %	unknown *
5	2	0.71 Rf	0.9 AU	0.74 Rf	40.7 AU	9.25 %	0.76 Rf	6.7 AU	762.9 AU	10.24 %	unknown *
5	3	0.81 Rf	1.6 AU	0.82 Rf	15.7 AU	3.56 %	0.85 Rf	0.7 AU	357.2 AU	4.79 %	unknown *
6	1	0.43 Rf	0.3 AU	0.44 Rf	32.8 AU	6.69 %	0.46 Rf	0.1 AU	313.4 AU	3.22 %	unknown *
6	2	0.64 Rf	4.7 AU	0.67 Rf	57.9 AU	93.31 %	0.73 Rf	6.0 AU	9426.7 AU	96.78 %	unknown *
7	1	0.64 Rf	4.1 AU	0.67 Rf	94.7 AU	00.00 %	0.73 Rf	6.3 AU	1994.2 AU	00.00 %	unknown *
8	1	0.47 Rf	3.2 AU	0.50 Rf	11.9 AU	2.25 %	0.53 Rf	0.6 AU	283.7 AU	1.81 %	unknown *
8	2	0.63 Rf	2.0 AU	0.67 Rf	17.8 AU	97.75 %	0.75 Rf	4.9 AU	5363.8 AU	98.19 %	unknown *
9	1	0.64 Rf	3.6 AU	0.66 Rf	07.9 AU	00.00 %	0.70 Rf	1.8 AU	1369.9 AU	00.00 %	unknown *
10	1	0.46 Rf	0.2 AU	0.49 Rf	11.3 AU	5.07 %	0.53 Rf	2.1 AU	309.2 AU	7.10 %	unknown *
10	2	0.64 Rf	7.2 AU	0.66 Rf	48.9 AU	66.93 %	0.71 Rf	3.9 AU	2613.1 AU	60.00 %	unknown *
10	3	0.76 Rf	0.2 AU	0.77 Rf	62.3 AU	28.01 %	0.83 Rf	0.6 AU	1432.7 AU	32.90 %	unknown *
11	1	0.64 Rf	1.0 AU	0.66 Rf	62.5 AU	48.00 %	0.71 Rf	5.6 AU	3030.0 AU	45.71 %	unknown *
11	2	0.73 Rf	4.6 AU	0.76 Rf	76.1 AU	52.00 %	0.80 Rf	2.3 AU	3599.2 AU	54.29 %	unknown *
12	1	0.62 Rf	3.0 AU	0.65 Rf	20.9 AU	7.66 %	0.65 Rf	5.3 AU	284.6 AU	3.88 %	unknown *
12	2	0.66 Rf	5.6 AU	0.71 Rf	19.1 AU	43.55 %	0.72 Rf	1.1 AU	3216.5 AU	43.90 %	unknown *
12	3	0.75 Rf	2.4 AU	0.77 Rf	33.5 AU	48.79 %	0.82 Rf	7.9 AU	3826.1 AU	52.22 %	unknown *

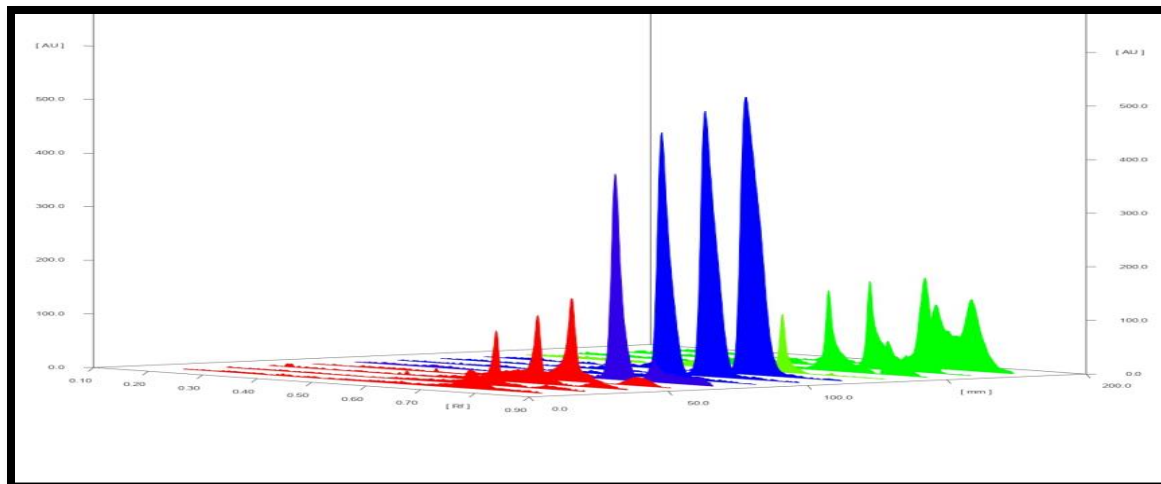


Figure 1: HPTLC Densitometric chromatogram of *Dalbergia Sissoo* aqueous extract @254.

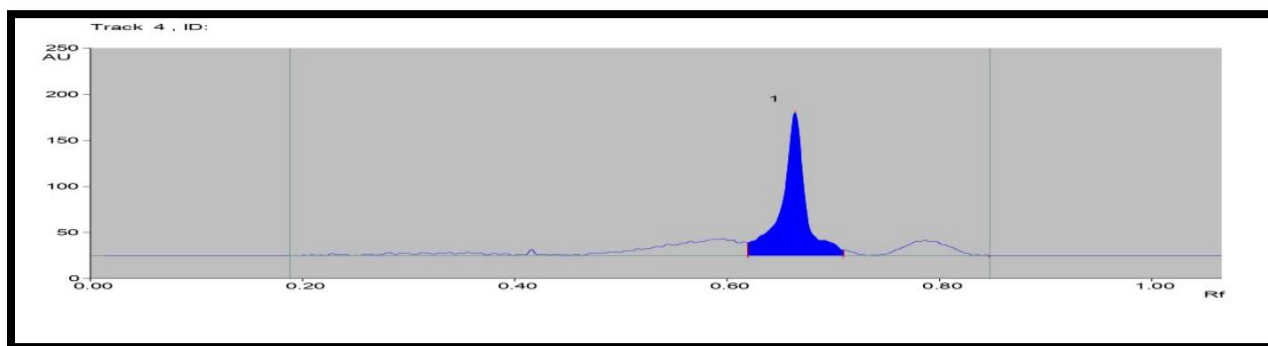


Figure 2: HPTLC Densitometric chromatogram of *Dalbergia Sissoo* ethanol extract @254.

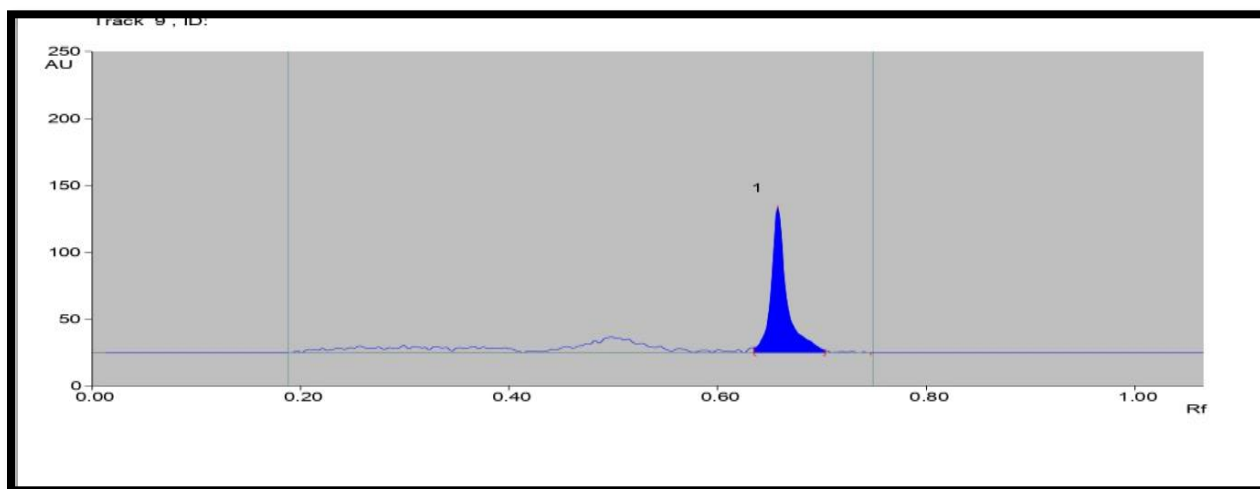
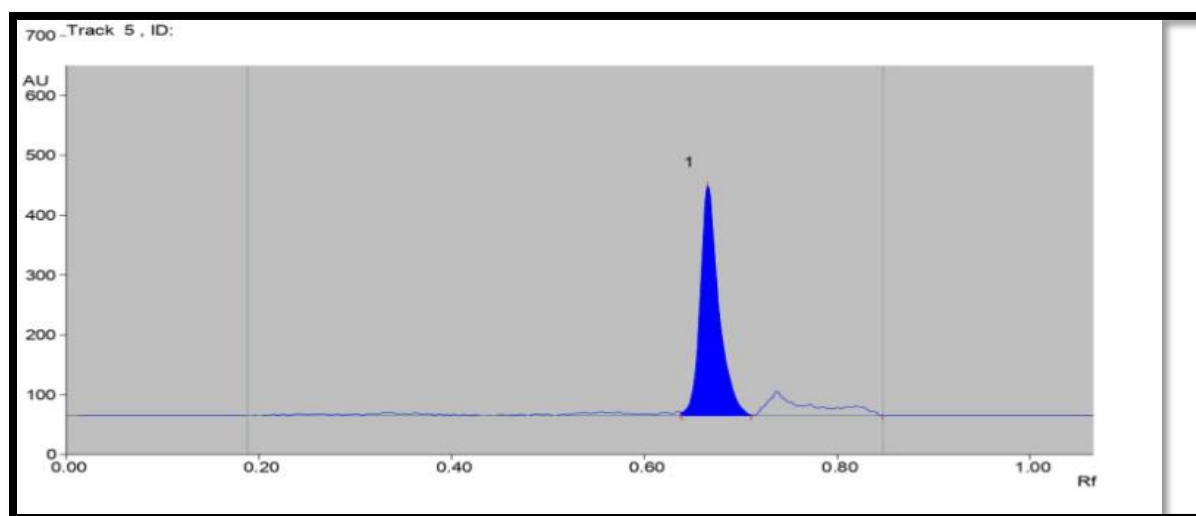


Figure 3: HPTLC Densitometric chromatogram of Phthalic acid@254.



### DISCUSSION:

The HPTLC finger print profile (densitometric scan) of aqueous, ethanolic leaf extract of Shimshapa & phthalic acid at UV 254nm are presented in figures 1 to 3. The densitometric scan at UV 254nm revealed the presence of 14 phytochemical components in both extracts with  $R_f$  value ranging from 0.04 to 0.95. It is clear from the chromatogram that the three components with  $R_f$  values 0.64, 0.64, 0.64 are present in higher concentrations with peak areas 6332, 2112.5, 2613.1 respectively for phthalic acid, aqueous extract & methanolic leaf extract of Shimshapa.

### CONCLUSION

Phthalic acid was present in aqueous leaf extract of *Shimshapa* and the  $R_f$  value was 33.26  $\mu\text{g/ml}$ . Phthalic acid was present in Ethanolic leaf extract of *Shimshapa* and the  $R_f$  value was 40.84  $\mu\text{g/ml}$ . The obtained data in the present investigation can serve as a standard for the identification of the drug *Shimshapa*. This will be helpful for the

identification of the plant & ensure the quality and safety of the drug.

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### REFERENCES

1. Kirtikar and Basu, Indian Medicinal Plants, 2<sup>nd</sup> revised edition 2012, Periodical expert book agency, Delhi, pg.no819.
2. Acharya Priyavathsharma yavam Dr.Guruprasad Sharma, *Kaiyadeva Nighantu, Aushadhi varga, sloka* no.677-679, 1<sup>st</sup> edition 1979, Chaukambha orientalia, Varanasi, pg no.180, 181.
3. Shri Bhavamishra, Dr. K.C Chuneekar (commentor), Dr.G.S Pandey(editor), *Bhavaprakasha Niganthu, Vatadi varga, sloka* no.24, 2004, Chaukambha Bharath academy, pg no.52-523.



4. Acharya Priyavatha Sharma and Dr.Guruprasada Sharma, Jayakrishna Ayurveda Granthamala *Dhanvantari Nighantu, Aamradi varga, sloka* no.111-112, 2<sup>nd</sup> edition, Chaukambha Orientalia, pg.no-169
5. Kirtikar and Basu, Indian Medicinal Plants, 2<sup>nd</sup> revised edition 2012, Periodical expert book agency, Delhi, pg.no-819.
6. Sudhakar et.al, Review article, International Journal of Research in Pharmacy and Chemistry, volume 3(2), 2013, ISSN: 2231-2781.
7. Anupam Kr Sachan, Review article, International Journal of Phytomedicines, volume 8, 2016, ISSN: 0975-0185.
8. Akita Jain et.al, Current Research in Pharmaceutical Sciences, 4(1), 2014, ISSN: 22502688.

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