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STANDARDIZATION OF *HARIDRADI LEPA* USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Introduction: Standardization is the process of evaluating quality & purity of drugs by means of various parameters. HPTLC is on among them which works on the principle of separation is adsorption. It is enhanced form of TLC. *Haridradi Lepa* is explained by our acharyas under *granthi/ arbuda chikitsa*. **Aims & Objectives:** To standardize and evaluate the different constituents present in *Haridradi Lepa*. **Methodology:** 3µl, 6µl, 9µl of each of the *Haridradi Lepa* extract was applied on a pre-coated silica gel plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate: Formic acid (5:1.5:0.5). The developed plates were visualized in short UV, long UV and then derivatized with vanillin sulphuric acid and scanned under UV 254, 366 nm and 620nm. R_f, color of the spots and densitometric scan were recorded. **Results:** Under short UV it showed 1 band, under long UV 9 bands were observed. Following derivatisation vanillin sulphuric acid (VSA) showed 3 bands. When the plates were densitometrically scanned at 254nm, it showed 6 bands, at 366nm, there were 5 bands observed, at 620nm 6 peaks were observed. **Conclusion:** The peaks observed in HPTLC can be considered for fingerprint profile analysis.

Keywords: *Haridradi Lepa, arbuda, granthi*, HPTLC.

1. INTRODUCTION

Standardization refers to the confirmation of its identity, determination of its quality & purity, detection of nature of adulterants by various parameters. It includes morphological, microscopical, physical, chemical & biological tests. Any product before marketed is necessary to undergo these assessments.

Haridradi Lepa is a herbo-mineral formulation. It has been explained under various classical textbooks under *granthi/ arbuda chikitsa* like in *Bhavaprakasha Nighantu*[1], *Gadanigraha*[2], etc. According to *Bhavaprakasha Nighantu* it comprises of *mulaka kshara*, *haridra churna* & *shankha churna* as ingredients.

HPTLC is an advanced form of Thin Layer Chromatography (TLC). It works on the principle of separation is adsorption. It has high resolution & accurate quantitative measurements than TLC. Hence an attempt has been made to standardize *Haridradi Lepa* using High Performance Thin Layer Chromatography.

2. AIMS & OBJECTIVES

To standardize and evaluate the different constituents present in *Haridradi Lepa*.

3. MATERIALS & METHODS[3]

3.1. Materials Required: Digital weight, water bath, pipette, alcohol (ethanol), toluene, ethyl acetate, formic acid, chloroform, dryer, vanillin-sulphuric acid spray, test tube, etc.

3.2. Instruments: Micro syringes, Linomat 5 applicator (sample applicator), pre-coated silica gel plate, twin trough chambers (hptlc development tank), scanner, UV cabinet visualization, digital camera for photo-documentation.

3.3. Stationary phase: Pre-coated silica gel plate.

3.4. Mobile phase: Toluene: Ethyl Acetate: Formic acid (5:1.5:0.5).

3.5. Sample preparation for HPTLC:

1gm of sample of water-soluble extractive of *Haridradi Lepa* is dissolved in 10ml of ethanol completely. This ethanol extract is taken in a test tube.

3.6. Procedure:

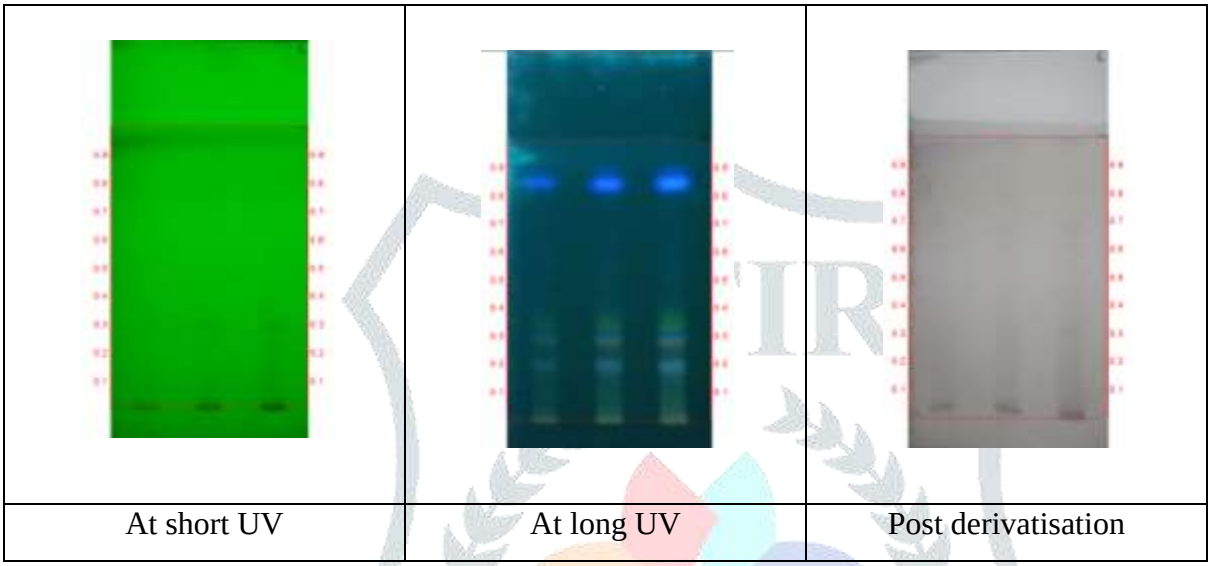
3µl, 6µl, 9µl of each of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl Acetate: Formic acid (5:1.5:0.5). The developed plates were visualized in short UV, long UV and then derivatized with vanillin sulphuric acid and scanned under UV 254, 366 nm and 620nm. R_f, color of the spots and densitometric scan were recorded.

4. RESULTS

Table 1: R_f values of sample of Alcoholic extract of *Haridradi Lepa*

At short UV	At long UV	Post derivatisation
-	0.20 (F. green)	-
-	0.27 (F. green)	-
0.30 (D. green)	0.30(F. green)	0.30 (purple)
-	0.33(F. green)	-

-	0.49(F. green)	-
-	0.56(F. green)	-
-	-	0.61 (Purple)
-	-	0.68 (purple)
-	0.77(F. green)	-
-	0.84 (F. blue)	-
-	0.90 (F. green)	-



At short UV	At long UV	Post derivatisation

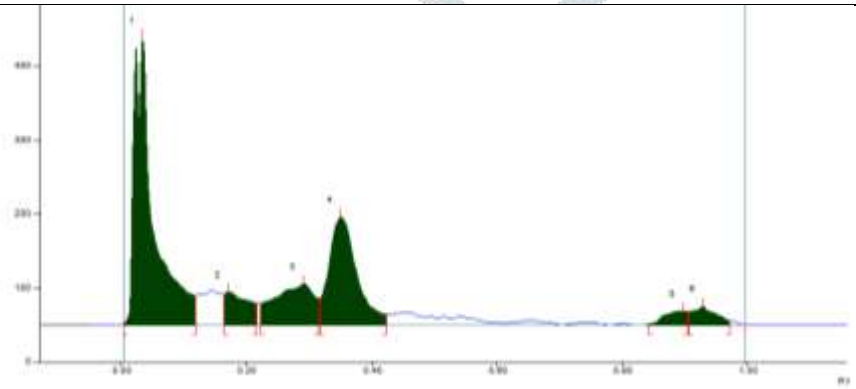
Figure 1: HPTLC Photo-documentation of Alcoholic extract of *Haridradi Lepa*

Solvent system – Toluene: Ethyl acetate: Formic acid (5.0:1.5: 0.5)

Track 1 – Ethanolic extract of Alcoholic extract of *Haridradi Lepa* – 3µl

Track 2 – Ethanolic extract of Alcoholic extract of *Haridradi Lepa* – 6µl

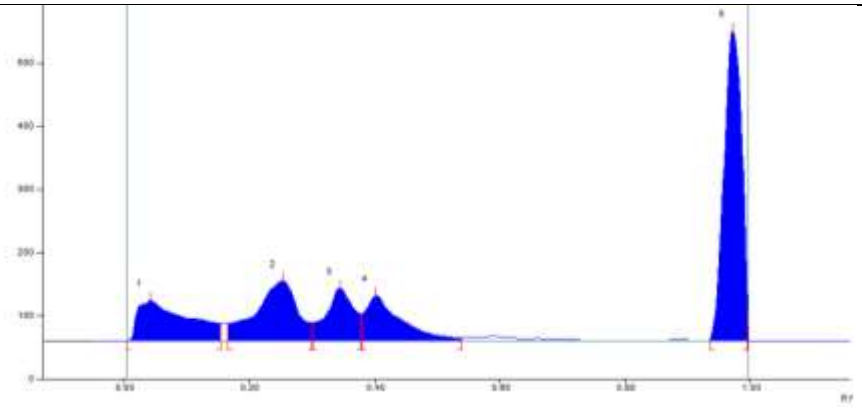
Track 3 – Ethanolic extract of Alcoholic extract of *Haridradi Lepa* – 9µ



Track 3, ID: Haridradi lepa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	1.4 AU	0.04 Rf	388.1 AU	57.10 %	0.12 Rf	40.1 AU	8707.6 AU	47.39 %
2	0.16 Rf	41.5 AU	0.17 Rf	44.9 AU	6.60 %	0.22 Rf	29.4 AU	1260.3 AU	6.86 %
3	0.22 Rf	29.5 AU	0.29 Rf	56.0 AU	8.24 %	0.32 Rf	35.7 AU	2526.2 AU	13.75 %
4	0.32 Rf	36.0 AU	0.35 Rf	146.0 AU	21.49 %	0.42 Rf	15.1 AU	4672.2 AU	25.43 %
5	0.84 Rf	1.7 AU	0.90 Rf	19.0 AU	2.80 %	0.91 Rf	18.4 AU	511.9 AU	2.79 %
6	0.91 Rf	18.4 AU	0.93 Rf	25.7 AU	3.77 %	0.97 Rf	5.4 AU	695.9 AU	3.79 %

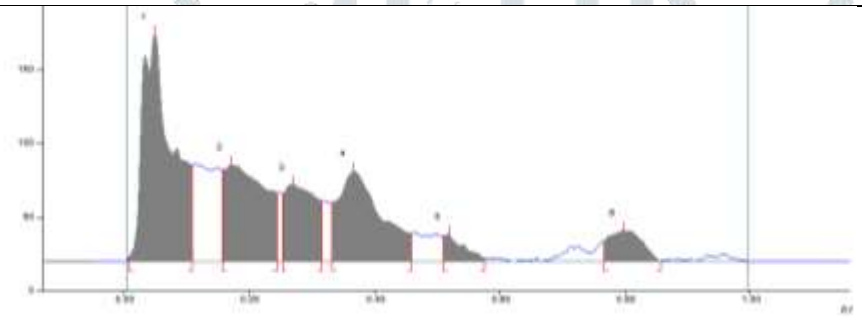
Fig 2a. At 254nm



Track 3, ID: Haridradi lepa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.2 AU	0.04 Rf	64.1 AU	8.01 %	0.15 Rf	27.9 AU	3667.5 AU	15.62 %
2	0.17 Rf	27.4 AU	0.25 Rf	93.7 AU	11.71 %	0.30 Rf	28.2 AU	4479.2 AU	19.08 %
3	0.30 Rf	28.3 AU	0.35 Rf	82.9 AU	10.35 %	0.38 Rf	42.0 AU	2594.0 AU	11.05 %
4	0.38 Rf	42.2 AU	0.40 Rf	70.9 AU	8.85 %	0.54 Rf	3.6 AU	2862.4 AU	12.19 %
5	0.94 Rf	1.0 AU	0.97 Rf	489.0 AU	61.08 %	1.00 Rf	49.3 AU	9871.8 AU	42.05 %

Fig 2b. At 366nm



Track 3, ID: Haridradi lepa

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	3.0 AU	0.05 Rf	153.6 AU	41.27 %	0.11 Rf	65.1 AU	5314.0 AU	36.30 %
2	0.16 Rf	62.0 AU	0.17 Rf	65.7 AU	17.66 %	0.25 Rf	46.5 AU	3115.1 AU	21.28 %
3	0.25 Rf	46.0 AU	0.27 Rf	52.2 AU	14.04 %	0.32 Rf	40.7 AU	1899.7 AU	12.98 %
4	0.33 Rf	39.5 AU	0.37 Rf	61.1 AU	16.42 %	0.46 Rf	18.8 AU	3078.1 AU	21.02 %
5	0.51 Rf	17.4 AU	0.52 Rf	18.3 AU	4.92 %	0.58 Rf	2.1 AU	420.8 AU	2.87 %
6	0.77 Rf	13.4 AU	0.80 Rf	21.2 AU	5.68 %	0.86 Rf	0.2 AU	812.7 AU	5.55 %

Fig 2c. At 620nm

Figure 2: Densitometric scan of Alcoholic extract of *Haridradi Lepa*

5. DISCUSSION

The chromatographic plate developed in toluene ethyl acetate (solvent system). When subjected to manual photo documentation at short UV showed 1band at Rf 0.30 (dark green), at long UV 9 bands were observed (different intensities of fluorescent green) namely at Rf 0.20, 0.27, 0.30, 0.33, 0.49, 0.56, 0.77, 0.84, 0.90. Following derivatisation vanillin sulphuric acid (VSA) showed 3 bands (all purple) at Rf 0.30, 0.61, 0.68. When the plates were densitometrically scanned at 254nm, it showed 6 bands. Among which major ones were at 0.29 (13.75%), 0.35(25.43%) were prominent ones. At 366nm, there were 5 bands observed, among which evident ones were at 0.25 (19.08%) and 0.97 (42.05%).

Following derivatisation at 620nm by densitometric scan 6 peaks were observed. Among which notable ones are at Rf 0.17(21.28%), 0.37 (21.02%) were major ones.

6. CONCLUSION

The peaks observed in HPTLC can be considered for fingerprint profile analysis. It can be used as standard markers of the sample of *Haridradi Lepa*. To analyze & interpret the results, it requires in detailed study with trial & error method.

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