

# CHROMATOGRAPHIC STANDARDISATION AND QUALITY VALIDATION STUDIES ON MARKET SAMPLES OF A TRADITIONAL DRUG

V. Sathyanathan<sup>1\*</sup> and P. Jayaraman<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Analysis and Quality Assurance,  
Tirumala College of Pharmacy, Bardipur (V),  
Dichpally (M), Nizamabad-503 230, Telangana, India.

<sup>2</sup>Plant Anatomy Research Centre, W. Tambaram,  
Chennai – 600 045, Tamil Nadu, India.

## ABSTRACT

An Indian Traditional drug 'AKIL' which is of controversial source was taken for analysis. Eight woods which were available in the name of AKIL in market were collected from various places and subjected for standardization studies with the authentic wood. In the present paper some plant metabolites were estimated, TLC and HPTLC profiles for all eight woods and the authentic sample were performed. Further anticancer marker was extracted from the authentic source. The specific amount of primary and secondary metabolites, the chromatographic finger printings and the chromatogram of the marker isolated for the authentic wood will help to substantiate the original identity from its market adulterants.

**Keywords:** Akil, Adulteration, Tannin, Colorimeter, TLC, HPTLC, UV, Liriodenine.

## INTRODUCTION

Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethnic medicines. Even today, plants are the most exclusive sources of drugs for the majority of world's population and plant products constitute about sixty percent of prescribed medicines<sup>1</sup>. The World Health Organization (WHO) estimates that 80% of the world's population still relies on herbal medicines as its major source of medicinal products<sup>2</sup>.

Today, the world over, there is a great deal of interest in Ayurvedic System of Medicine and thus the demand for various commonly used medicinal plants in the production of Ayurvedic medicines is ever increasing. Due to varied geographical locations where these plants grow, coupled with the problem of different vernacular names, these plants are known, a great deal of adulteration or substitution is encountered in the commercial markets.

Therefore, reproducible standards of each plant are necessary for effective Quality Control. The most important factor, which stands in the way of wider acceptance of herbal drugs, is the paucity of standard protocols for checking the genuineness, purity and quality of the raw drugs that are used in different formulations. The World Health Organisation (WHO) in a number of resolutions has emphasized the need to ensure quality control of herbal drugs by applying of suitable standards including modern techniques. WHO has also issued "Guidelines for Quality Control Methods for Medicinal Plant Materials"<sup>3</sup>.

Many herbal drugs seem to remain lacking authentic diagnostic parameters in the event of checking their identity. *Akil* or *Agaru*, a renowned incense and medicinal wood of considerable antiquity, is one of the drugs that need scientific evaluation. The agil wood, *Aquilaria malaccensis* Lam. Syn. (*A. agallocha* Roxb.) of *Thymelaeaceae*<sup>4</sup> family is very costly and of rarity. So it is adulterated

by various other woods in trade. The primary aim of the present study is to make a comprehensive survey of the woods that are sold in the markets in the name of Akil and to investigate the original *Akil* on the basis of available data and direct investigation. Few scattered information is available from the literature on the chemical compounds of various woods that are proclaimed as Akil. It is planned to make more extensive phytochemical study of the wood samples.

All the wood samples which are claimed as Akil by the raw-drug dealers will be subjected to phytochemical evaluation and chromatographic standardization. These studies will include estimation of primary and secondary metabolites, extraction of a chemical marker from the authentic source, TLC and HPTLC profiles. The results of the study will enable to isolate the Akil from the adulterants and substitutes. The present study is believed to throw significant light on the scientific standardization of the time-renowned drug.

#### MATERIALS AND METHODS

Eight wood samples sold in the name of Akil were procured from Chennai, Dehradun and Delhi markets. Authentic wood sample of *A. malaccensis* was authenticated by Prof. P. Jayaraman, Director, PARC, W. Tambaram, Chennai. These samples are named as S I – S IX in present study and the authentic drug is named as S I. The voucher specimens for all wood samples studied are deposited in PARC, Chennai.

The solvents were purified and standardized and used for carrying out the experiments. The solvents like n-Hexane, Toluene, Chloroform, Ethanol, Methanol, Benzene, Ethyl acetate were purified as per standard procedures<sup>5</sup>.

The samples were subjected to **a.** estimation of plant metabolites, **b.** extraction of liriodenine, **c.** TLC and **d.** HPTLC finger printings for standardizing the samples and to evaluate the biological active chemicals responsible for their therapeutic efficacy.

##### **a. Estimation of Plant Metabolites**

The various plant metabolites were studied for all the nine samples under study. The metabolites were estimated using UV/Visible spectrophotometer (PERKIN ELMER LAMBDA EZ201) so as to keep the parameters as standards and to support the chemical potency and therapeutic efficacy for the plants studied.

- i. Total Carbohydrates for all the nine samples of Akil were estimated by Anthrone method<sup>6</sup>.

- ii. Total Polysaccharides for the samples were found out by the procedure followed as per standard texts<sup>7,8</sup>.
- iii. Estimation of Proteins was carried out by the prescribed method<sup>9</sup>.
- iv. Tannins in nine market samples of Akil were estimated employing colorimetric method. Estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungsto molybdic acid by tannins in alkaline solution<sup>10</sup>.

##### **b. Extraction of Liriodenine from *A. malaccensis***

Liriodenine, an oxoaporphine alkaloid, an anticancer chemical marker compound was extracted from authentic sample of *A. malaccensis* (S I). The heartwood of the plant was extracted successively with chloroform and acetone after defatting with hexane. The alkaloid was isolated from the extract by chromatography on activated alumina. Liriodenine was obtained by eluting with chloroform. The compound was recrystallised from large volumes of chloroform as yellow needles<sup>11</sup>. The melting point of the compound was found out using standard procedures<sup>12</sup>.

##### **c. Thin Layer Chromatography**

Chromatographic procedures were used as a method of separating and isolating the plant constituents. The technique was originally developed by the Russian botanist 'Tswett' in 1906 during the course of an investigation into the nature of leaf pigments. Thus it is essentially a group of techniques for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is moving past the other. The principle behind the separation of the compounds was adsorption at the solid-liquid interface. When a mixture of compounds are spotted on a TLC plate, the compound which is not strongly adsorbed moves up readily, along with the solvent, those which are more strongly adsorbed moves up less readily leading to the separation of the compounds<sup>13,14,15</sup>. TLC for all the nine samples and Liriodenine were carried out under U.V. 254 and 366nm.

##### **d. HPTLC Finger Printings**

In the last decade HPTLC emerged as an important tool for drugs and formulations. This includes developing TLC finger print profiles and estimation of chemical markers and biomarkers<sup>16, 17, 18, 19</sup>.

Various steps involved in TLC/HPTLC/ planar chromatography.

- i. Selection of TLC/HPTLC plates and solvent.
- ii. Sample preparation including any clean up and pre-chromatographic derivatization.
- iii. Application of sample.
- iv. Development (Separation), Detection including post-chromatographic derivatization.
- v. Quantitation
- vi. Documentation

### Sample Preparation

The coarsely powdered drug 4g was soaked in AR HPLC grade methanol (50ml) for 18 hours boiled and filtered. The filtrate was

concentrated and made up to 10 ml in a standard flask. The remaining drugs (S II -SIX) were extracted adopting similar procedure.

### Development Method

The samples (10 µl) were applied on aluminium sheets precoated with silica gel (Merck) 60 F<sub>254</sub> with 0.2 mm layer thickness using Camag Linomat IV sample applicator. Mobile phase used for developing the chromatogram was Toluene: Ethyl acetate (5.0:1.5). The plate was scanned using Camag densitometer Scanner 3V 1.13 equipped with Cats V 4.04 software at 254nm using deuterium lamp<sup>14,15</sup>.

The Chemical Marker 'Liriodenine' was calculated using the formula:

$$\% \text{ of Marker Content in Extract} = \frac{\text{Area of Test}}{\text{Area of Standard}} \times \frac{\text{Concentration of Standard}}{\text{Concentration of Test}} \times 100$$

## RESULTS

### a. Estimation of Plant Metabolites

The estimation of the plant metabolites gave the following ranges for the nine samples: Carbohydrates (4.33-12mg%), Polysaccharides (11.4-28%w/w), Proteins (3.35-4.7%w/w) and Tannins (0.08-1.679mg%). S VI showed more yield for sugar and in S III, the carbohydrate content was found to be less compared to all other samples. Polysaccharides content was more in S IX. All the samples contained moderate yield of protein. S VI showed more tannins of all the samples collected. Both S VI and *A. malaccensis* yielded good percentage of Tannin, Sugar and Protein which are rich bioactive principles (Table - 1) and (Graphs 1 - 4).

### b. Extraction of Liriodenine

The heartwood of the plant was extracted successively with chloroform and acetone after defatting with hexane. Liriodenine was isolated from the extract by chromatography on activated alumina. The compound was obtained by eluting with chloroform and recrystallised from large volumes of chloroform as yellow needles. Its melting point was found to be 274 - 5°.

### c. Thin layer chromatography

TLC profile for the chloroform extracts developed in Toluene(5):Ethylacetate (1.5) of all the nine samples (S I to S IX) and Liriodenine were observed under UV 254 and 366 nm and presented (Fig. 1).

### d. HPTLC Finger Printings

HPTLC chromatogram of the methanol extract of each sample is summarized. In the HPTLC finger print of the S I, there were 2 spots of which peak with Rf 0.50 were in major yield whereas peak No.2 with Rf 0.74, is in poor yield. In S II there were 3 peaks of Rf 0.13, 0.52 and 0.97, whereas S III and S IV spot 3, with Rf 0.97, were in moderate yield, showed the absence of active chemicals. In sample S V, there were 9 spots. Peak No.2, 6 at Rf 0.14 and 0.51 were in good yield. Peak No.7 with Rf 0.75 is in moderate yield. In S VI there were four spots of Rf 0.07, 0.14, 0.50, 0.72 in which peak 3, Rf 0.5 was in moderate yield and the others were in poor yield. In S VII, there were 10 peaks of which peak 9, 10 of Rf 0.82, 0.87 were in major yield and peak 2, 3, 4, 5 and 7 were in moderate yield remaining peaks, 1,6,8 were in lesser quantities. In S VIII, there were 7 spots where peak 1, 4, 5, 7 were in moderate yield and 2, 3 and 6 were in milder yield. In sample S IX, there were 6 peaks of which peak 1, 6 of Rf 0.05, 0.77 were in major yield and peaks 2, 3, 4, and 5 of Rf 0.08, 0.11, 0.36, 0.48 were in moderate yield. The results are presented (Fig. 2). Liriodenine showed peak with Rf 0.5 and it was calculated to be 0.39% in S I. (Fig. 3)

S I and S V contained peaks with same Rf 0.50, 0.51. Super imposable UV spectra for the peaks showed 274 nm from the graph (Fig. 4).

## CONCLUSION

Phytochemical profiles showed similar type of compounds especially tannins, polyphenols, carbohydrates and proteins. The plant metabolites were found in good yield in S VI and

**A. malaccensis.** TLC profiles for all the samples showed marked differences in pattern under UV 264 and 366 nm. HPTLC finger printings showed differences in all samples which would be very useful in identifying the genuine drug chemically and other sources scientifically. An alkaloid liriodenine, a marker compound was isolated from **A. malaccensis** with m.p. 274 – 5° and was estimated. It would be useful in substantiating for the identification of genuine drug chemically. Attempts have made to identify

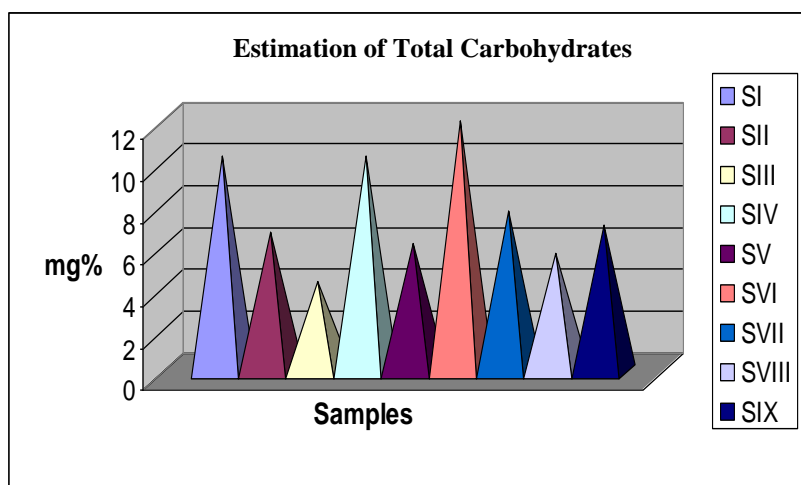
all the other samples (SII – SIX) which will be narrated in next paper.

#### ACKNOWLEDGEMENT

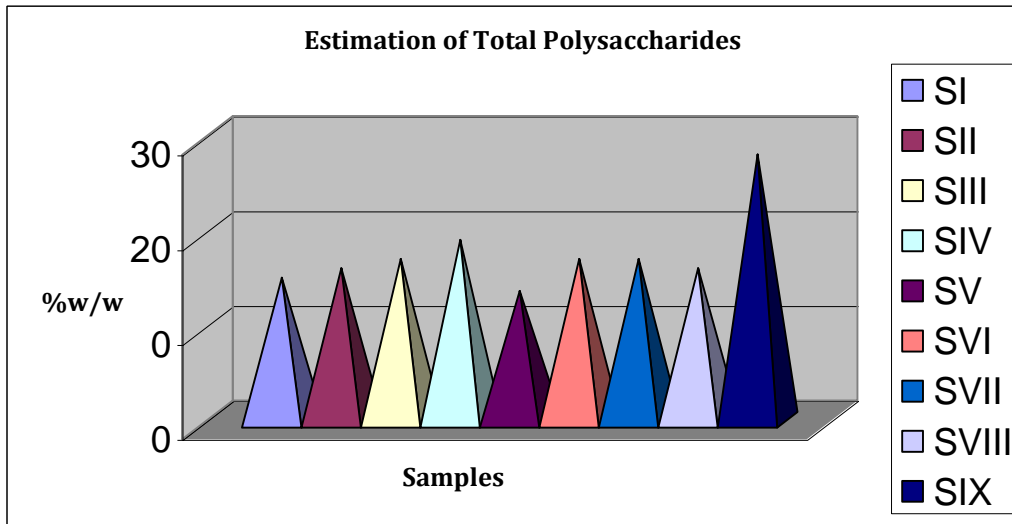
I acknowledge my thanks to Prof. Jayaraman, Director, PARC, W. Tambaram, Chennai, for authenticating the original sample and my deepest gratitude for late DR. A. Saraswathy, my guide, Director, CSMDRIA, Arumbakkam, Chennai for giving the facilities to carry out the chromatography work for all the samples.

**Table 1**

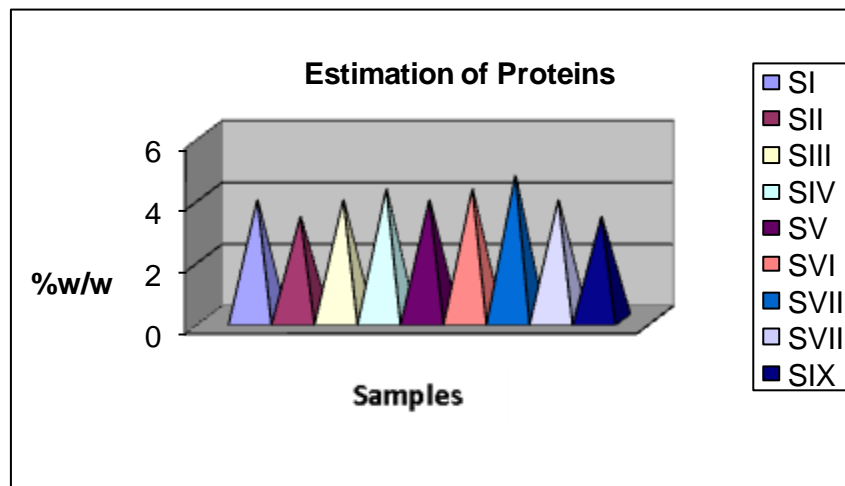
Samples	Carbohydrates (mg %)	Polysaccharides (%w/w)	Proteins (%w/w)	Tannins (mg%)
S I	10.33	13.6	3.90	0.759
S II	6.66	16.0	3.35	0.1573
S III	4.33	17.0	3.90	0.2997
S IV	10.33	19.0	4.25	0.2199
S V	6.16	11.4	3.90	0.405
S VI	12.00	17.0	4.25	1.679
S VII	7.66	17.0	4.70	0.998
S VIII	5.66	16.0	3.90	0.4148
S IX	7.00	28.0	3.35	0.08



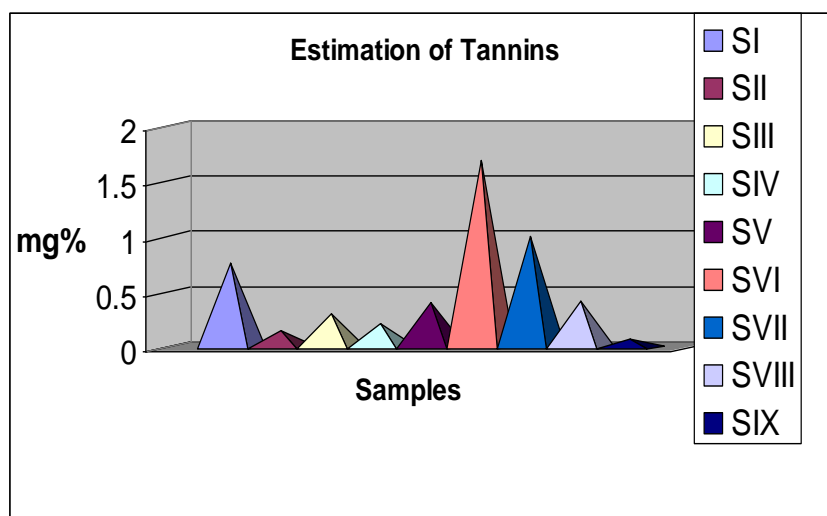
**Graph 1**



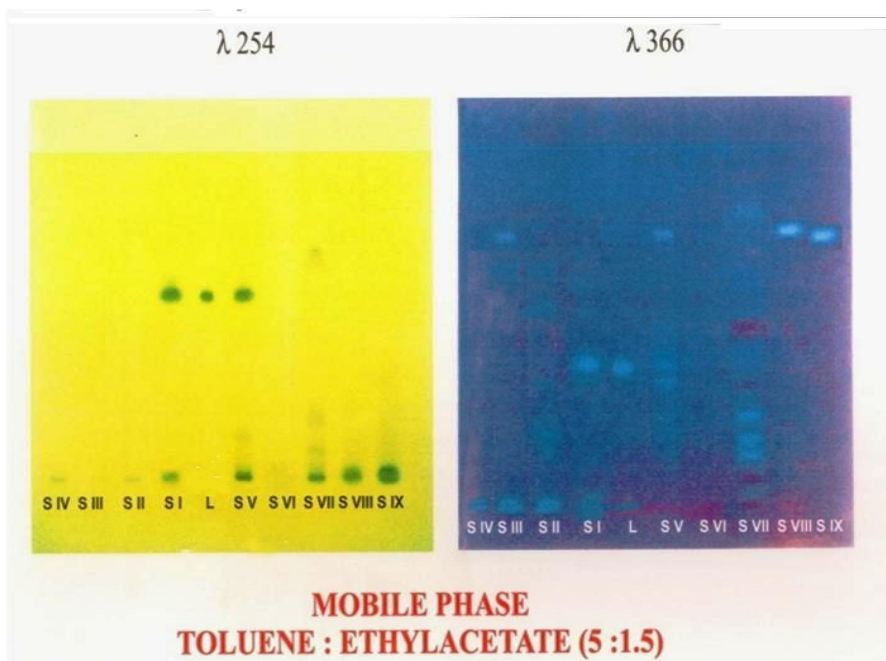
Graph 2



Graph 3

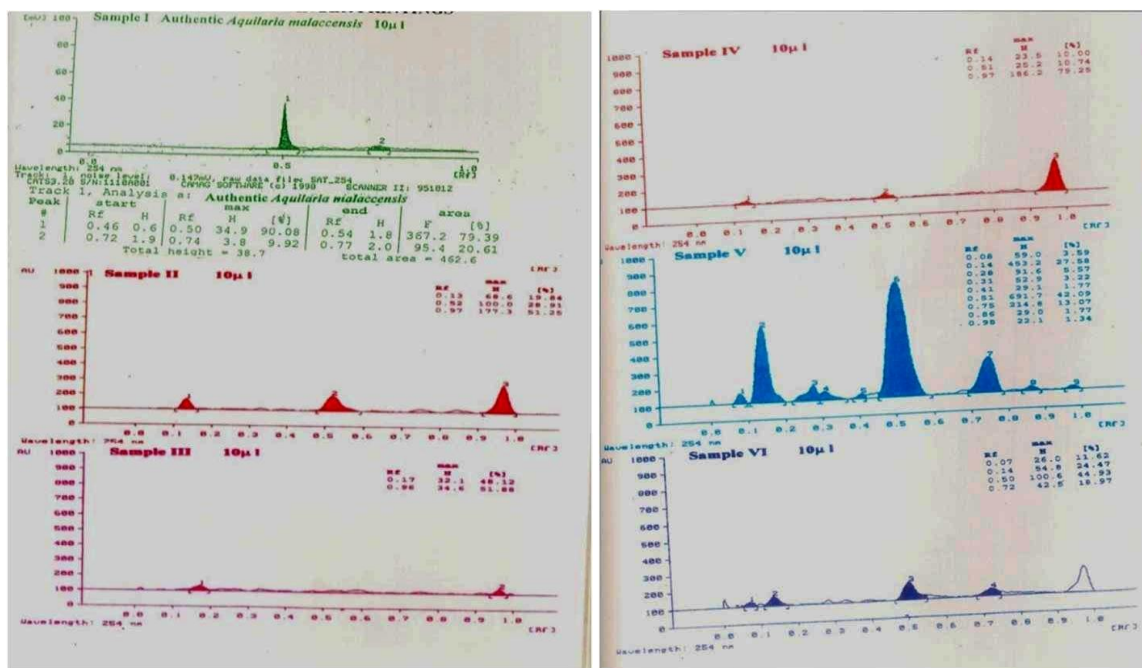


Graph 4



**S I - *A. malaccensis*(authentic wood)  
L - Liriodenine (Marker compound extracted from authentic wood)**

**Fig. 1: TLC profile of all the samples and marker 'L'**



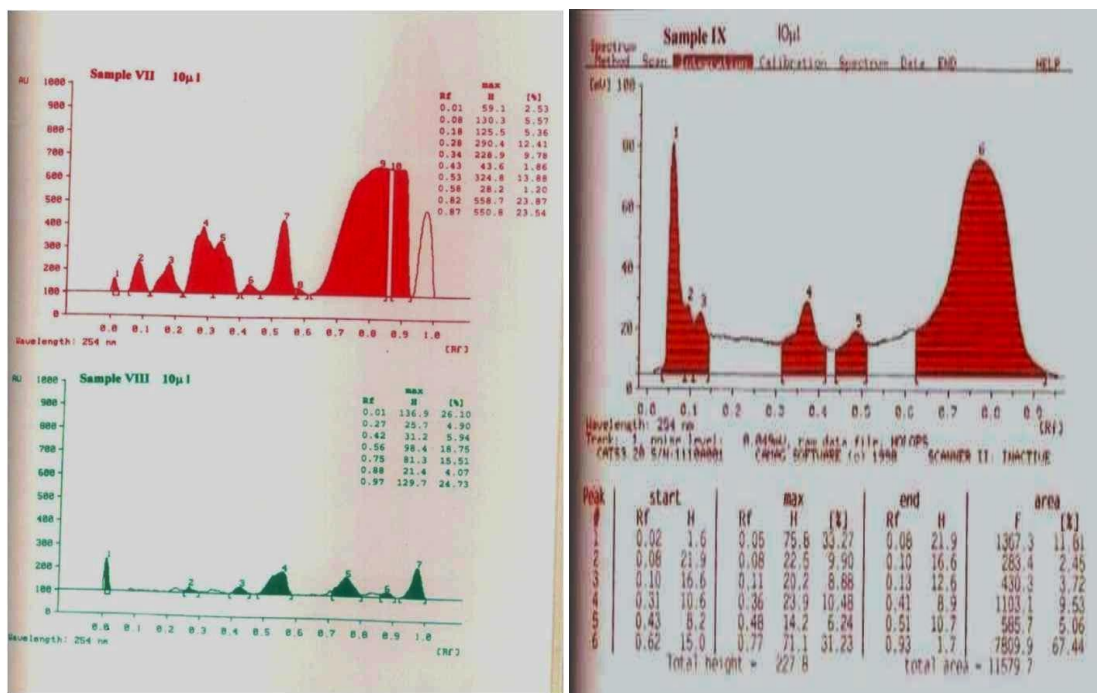


Fig. 2: HPTLC profiles of all the nine samples

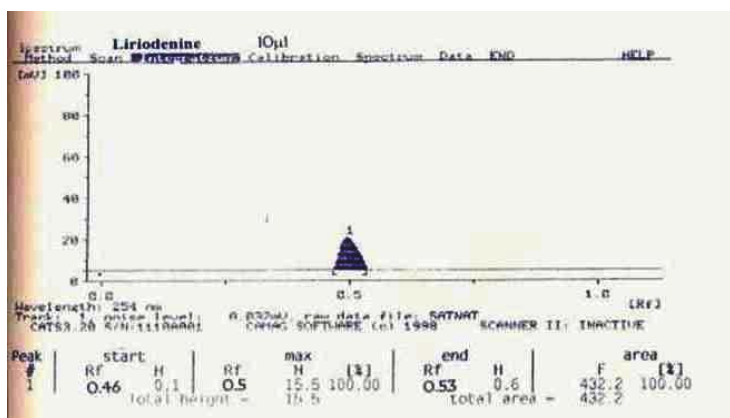


Fig. 3: HPTLC profile of liriodenine extracted from S I

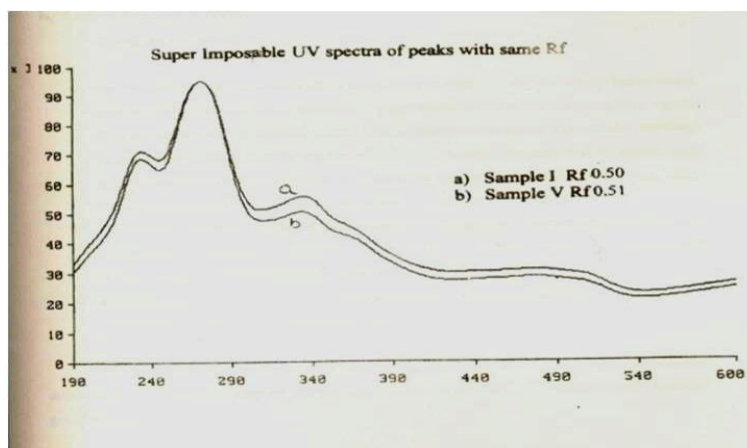


Fig. 4: Super imposable uv spectra of peaks with same Rf (S I & SV)

## REFERENCES

1. Farnsworth MR and Bingel AS. *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity*. 1977, Springer, New York, 61-73.
2. SukhdevHanda S. *Indian Herbal Pharmacopoeia*, Government of India, CSIR, 1999, Published by Indian Drug Manufacturer's Association and Regional Research Laboratory, India.
3. WHO, *Quality Control Methods for Medicinal Plant Materials*, 1998, Geneva, 10-31.
4. Mabberley DJ. *The Plant Book*, 2005, Press Syndicate of the University of Cambridge, U.K., 49.
5. Linkens HF and Jackson JF. *Modern Methods of Plant Analysis*. Adelaida and Erlangen. 1987;104 - 105.
6. Hedge JE and Hofreiter BT. *Carbohydrate Chemistry*. Academic Press, New York. 1962;40.
7. Dubois M, Gilles KA, Hamilton JK, Robers PA and Smith F. Phenol - Sulphuric Acid Method for Total Polysaccharides. *Anal Chem*. 1956;26(200):350.
8. Krishnaveni S, Thenmalai M and Balasubramanian S. Phenol Sulphuric Acid Method. *Food Chem*. 1984;15:229.
9. Lowry OH, Rosebrough NJ, Farr AJ and Randall RJ. Protein Measurement with the Folin-Ciocaltaeus Reagent. *J Biol Chem*. 1951;265 - 275.
10. Ranganna S. *Manual Analysis of Fruit and Vegetable Products*, 1977, Tata McGraw Hill Publishing Co.Limited, New Delhi, 69 - 70.
11. Natarajan RK, Natarajan M and Purushothaman KK. Alkaloids from Agar. *Bull Med Ethnobot*. 1983;4(1-2):81-84.
12. *Indian Pharmacopoeia*, Government of India, Ministry of Health and Family Welfare, 1996, Controller of Publications, Delhi, 2, A-53, 54, A-89, 947 - 949.
13. Lala PK. *Thin Layer Chromatography - Practical Pharmacognosy*, 1981, Liner, India, 9-32.
14. Wagner H, Bladt S and Zgainski EM. *Plant Drug Analysis - A Thin Layer Chromatography Atlas*, Springer-Verlag Berlin Heidel Berg, New York, Tokyo. 1995;51-90,291 - 298.
15. Sethi PD. *HPTLC - High Performance Thin Layer Chromatography, Quantitation Analysis of Pharmaceutical Formulation*, 1996, CBS Publishers and Distributors, New Delhi, India, 120.
16. Houghton P. High Performance Thin Layer Chromatography, *Journal of Pharmaceutical News*, 1999;6:21.
17. Lee KM and Mardersian DA. High Performance Thin Layer Chromatography. *J Pharm Sci*. 1981; 70:89.
18. Rajani M, Ravishankra MN, Srivastava N and Padh H. High Performance Chromatography. *J Planar Chromatography*. 2001;14:34.
19. Shah SA, Ravi Shankar MN, Nirmal A and Shisla Suhagia BN. *J Pharm Pharmacol*. 2000;52:445.