HPTLC FINGERPRINT PROFILE OF ROOT TUBER OF SMILAX CHINA

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ABSTRACT

Objective : To develop the finger print of medicinally and economically important root tuber of Smilax china Method: Methanolic extract of the root developed in mobile phase of Toluene:Ethylacetate:Formic acid(3.5:5.5:1) using standard procedures and scanned under UV at 254nm, 500nm. Result: HPTLC finger printing of Methanolic extract has shown several peaks with different Rf values. Conclusion: Since there is no previous hptlc study on this drug, this fingerprint would help in the identification and authentication of this species and provide referential information for standardization of the Smilax china for curing many diseases

Keywords : HPTLC Finerprinting , Methanolic Extract , Smilax China

I.INTRODUCTION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components [1].

The quantity and quality of phytochemicals present in plant parts may differ from one part to another. In fact, there is lack of information on the distribution of the biological activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [2].

Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world [3]. In the search for phytochemicals that may be of benefit to the pharmaceutical industry, researchers sometimes follow leads provided by local healers in a region [4]. Following such leads, plant parts are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product.

The recent advances in modern medicine have evolved from folk medicine and traditional system by probing through the various phytochemical and pharmaceutical screening is necessary for the evolving trends in modern medicine. It has estimated that 56% of lead compounds for medicines in British national formulary and natural products [4].

Standardization of plant material is essential for identification and quantification of active constituents in the plant material for drug formulation using chromatographic and spectral fingerprints.HPTLC methods are more proficient, faster and outcomes are more reliable and reproducible. When combining HPTLC with digital scanning profiling, It also provides accurate Rf values and quantifiable analysis of samples by in situ scanning densitometry aided by the formation of easily detective derivatives by post chromatography chemical reactions as required as well as record of separation in the form of chromatography with fractions represented as peaks with defined parameters including observance (Intensity), Rf height and area [5]. The pictorial fluorescence image of HPTLC coupled with digital scanning profile is more attractive to herbal analysts for constructing an herbal chromatography fingerprint for means ofHPTLC [6], [7].

1.1 Smilax China

Smilax Chinensis L (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south indian states namely Andrapradesh , Tamilnadu and Karnataka[8],.several species of Smilax are well known Chinese traditional medicines used as anti-inflammatory, anti-oxidants , anti-cancer and analgesic agents. The tubers of Smilax chinesis have widely used used in Chinese traditional medicines for treatment of diverse disease, especially for pelvic inflammation and chronic pelvic inflammation [9].

II.MATERIALS AND METHODS

2.1. Instrumentation

A Camag HPTLC system, HPTLC aluminium sheet silica gel (E-Merck), CamagLinomat V, Camag TLC scanner, Camag Visualizer, Win cats software.

2.2. Materials Andreagents

HPLC grade methanol were purchased from E-merk , India.

2.3. Plant Material

Smilax china were collected from the forest in Tirunelveli, District, Tamil Nadu, India and authenticated by National Institue of Siddha Medicine , Department of Botany , Chennai , India the root tuber were dried leaves and powdered that was stored in air tight container.

2.4 Preparation Of Methanolic Extract

The course powder weighed accurately 750gms was soaked in n-hexane for defatting for 48 hours and then

successively extracted in methanol at room temperature, the solvent was then removed by filtration and fresh solvent was added to the plant materials, the extraction process was twice repeated the combined filtrate were then evaporated under reduced pressure to give a dark green Viscous Mass the extract was stored at 0-4^{o}C, 20% yield was acquired[10].

2.5. Chromatography

10µ Aliquots of the extract separately applied on silica gel 60 F254 pre-coated HPTLC plates (5x10 cms) and desiccator used to stockpile the plates Hamilton micro syringe usedfor the purpose of application and the same was mounted.Everything tested using Camag Linomat-V applicator included in the Camag HPTLC systemprogram of the Wincats Software .

Spotting was done on the TLC. Ascending development of the plate, the migration distance 85mm (distance to the lower edge was 5mm) was performed at 25 ± 20^{o}C with Toluene:Ethylacetate:Formic acid(3.5:5.5:1) as a mobile phase in a Camag chamber previously saturated for 30 minutes. 10µl concentration of the samples was applied in three Tracks as 8mm bands at spraying rate of 150nl/s. After development the plate was Air-dried.

Densitometry scanning was then performed with a Camag TLC scanner equipped with Win-Cats software version 1.3.0 at λ_{max}=254nm and 366nm the slit dimensions were 6.00 x 0.03 mm micro and the chromatogram were Recorded.

III. RESULTS

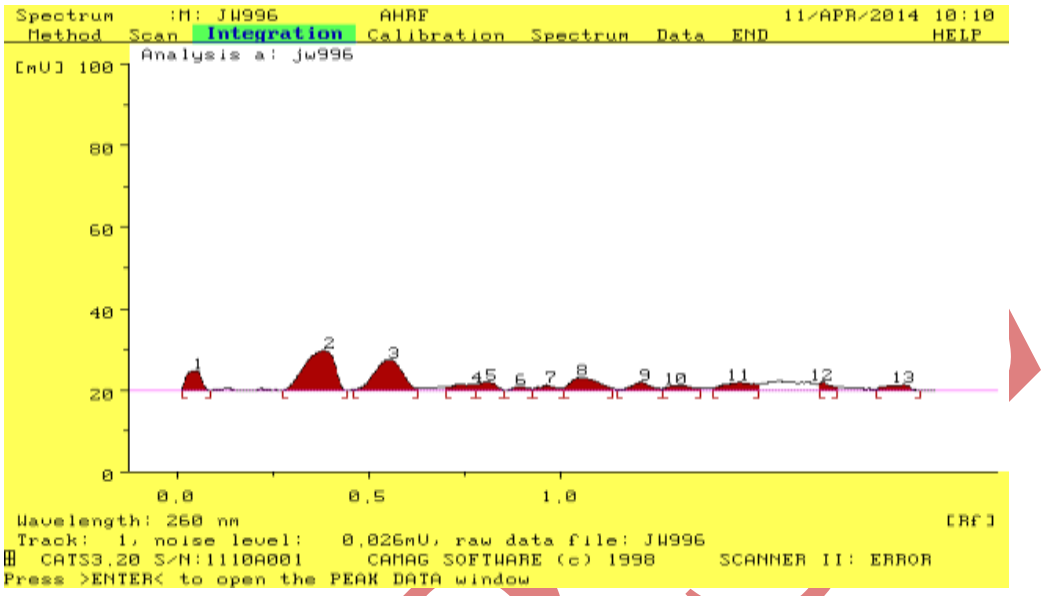
The Methanolic extracts of Smilax china were subjected to HPTLC analysis by specific solvent system Toluene:Ethylacetate:Formic acid(3.5:5.5:1) and detected under UV at 254nm(Fig.1) and 500 nm(Fig.2).

Fig.No.1.HPTLC (FINGER PRINT) CHROMATOGRAM OF METHANOLIC EXTRACT EXTRACT OF SMILAX CHINA AT 254NM

Fig.No.2.HPTLC (FINGER PRINT) CHROMATOGRAM OF METHANOLIC OF SMILAX CHINA AT 500NM

GRAPH 1: 10µl of METHANOL EXTRACT OF SMILAX CHINA ,

AT APPLICATION POSITION12mm,25mm, 38mm at 254nm RESPECTIVELY



The HPTLC images shown in Graph. 1 indicate all the sample constituents were clearly without any tailing and diffuseness

Table1. Peak List And Rf Values Of The Chromatogram of 10 µl Of METHANO Extract Extract Of Smilax China At 254nm

S. No	Rf	Height	Area	Lambda max
1	0.03	4.8	76.0	200
2	0.38	9.8	311.7	200
3	0.55	7.5	221.7	200
4	0.77	1.5	35.0	200
5	0.81	2.0	38.7	395
6	0.88	1.0	15.3	384
7	0.96	1.4	22.7	387
8	1.05	3.1	93.3	288
9	1.21	2.0	41.8	387
10	1.30	1.2	32.4	311
11	1.47	2.0	66.0	394
12	1.69	2.0	22.7	393
13	1.90	1.4	36.4	338

It is evident from the table the maximum Rf of smilax china is at 0.38 and 0.55

GRAPH.No.2: HISTOGRAM DERIVED WITH VANILLIC SULPURIC ACID

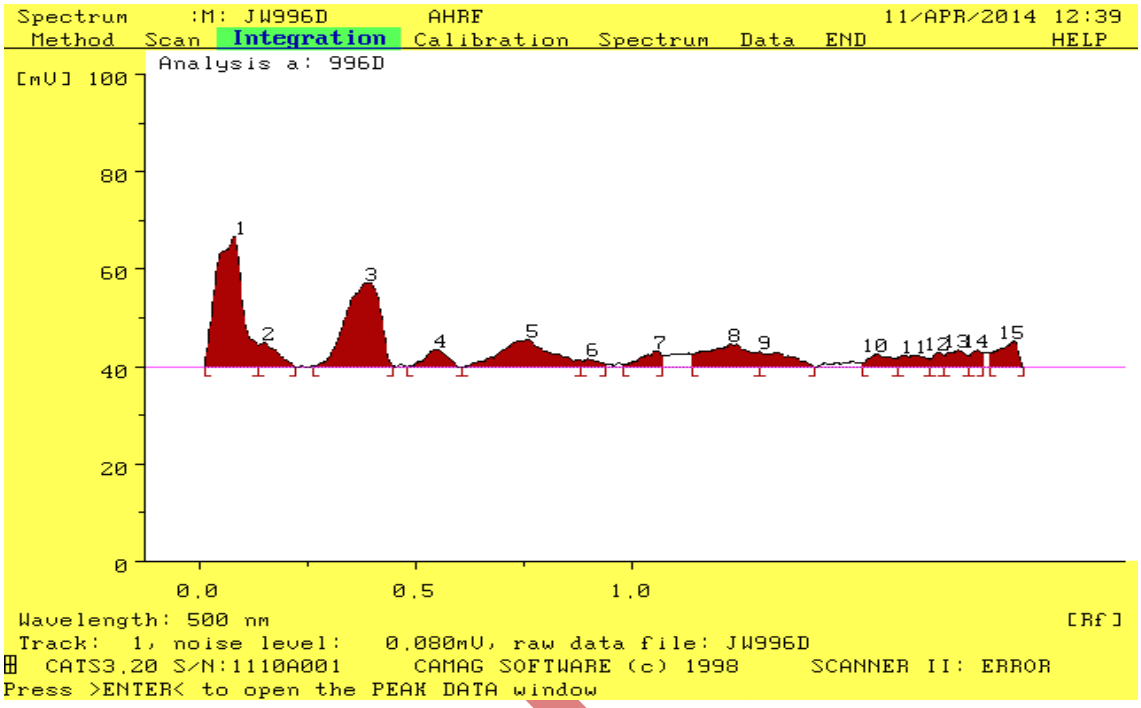


TABLE.NO.2 PEAL LIST AND Rf VALUES OF THE CHROMATOGRAM OF VALUES 10 µl of METHANOL EXTRACT OF SMILAX CHINA AT 500NM

It is evident from the table the maximum Rf of Smilax china is at 0.07 , 0.38 and 0.54nm respectively same as obtained in 254nm

S. No	Rf	Height	Area	Lambda max
1	0.07	26.8	661.9	526
2	0.14	5.1	88.3	768
3	0.38	17.3	566.5	510
4	0.54	3.7	76.4	548
5	0.75	5.7	273.1	548
6	0.89	1.7	25.7	548
7	1.05	3.3	61.5	548
8	1.23	4.7	195.7	548
9	1.34	3.2	93.7	548

10	**1.57**	**2.7**	**56.1**	**548**
11	**1.66**	**2.4**	**58.2**	**548**
12	**1.71**	**2.9**	**27.9**	**548**
13	**1.76**	**3.5**	**59.2**	**548**
14	**1.81**	**3.5**	**36.7**	**548**
15	**1.89**	**5.3**	**96.6**	**548**

IV. DISCUSSION

Thus the HPTLC chromatogram developed for Smilax china methanolic extract with specific Rf values serve as a better tool for standardization of the plant drug. Characteristic HPTLC finger printing of particular plant species will help in the identification and quality control of a particular species, and provide basic information for isolation, purification of various compounds and drug development for various disease condition.

V. CONCLUSION

HPTLC is the most reliable method for development of chromatographic fingerprints to determine major active constituents of medicinal plants, HPTLC analysis of root tuber of Smilax china can provide standard fingerprint and can be used as a reference for the identification and quality control of the drug

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