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HPTLC FINGERPRINT OF *ZIZIPHUS MAURITIANA* Lam. (FRUIT) EXTRACT

Dr. Sukhjeet Kaur Gujral*

Department of Chemistry, Guru Nanak Institute for Research & Development, Shiromani Gurudwara Prabandhak Committee‟s Guru Nanak Khalsa College, Matunga, Mumbai – 400019.

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***Corresponding Author**
Dr. Sukhjeet Kaur Gujral
Department of Chemistry, Guru Nanak Institute for Research & Development, Shiromani Gurudwara Prabandhak Committee‟s Guru Nanak Khalsa College, Matunga, Mumbai – 400019.

ABSTRACT

***Ziziphus mauritiana* Lam**. Commonly called as ‘Ber’ is widely distributed throughout India. The fruit has potential health benefits and medicinal properties. Global interest in the use of medicinal and aromatic plants is increasing. Utilization of natural products is radically changing. Standardization of these fruit samples needs potent analytical tools. HPTLC as a versatile tool for the analysis of fruit sample was selected which was used for development of HPTLC fingerprints analysis and for standardization of *Ziziphus mauritiana* Lam**.** (fruit) extract. In the present research article, HPTLC fingerprint profile of raw material of *Ziziphus mauritiana* Lam. (fruit) using different extracts was developed and this method was used for the separation of phytochemical constituents for *Ziziphus mauritiana* Lam. (fruit) extracts. HPTLC of these extracts was performed on silica gel 60F 254 by semi-automatic sample applicator and using solvent system. Methyl alcohol: Toluene: Ethyl acetate (4.0: 6.0:1.0) v/v/v. Hence, it was concluded that established fingerprint provides theoretical and technical support for routine quality control, species identification, authentication and was appropriate for standardization of drug.

KEYWORDS: Camag HPTLC, Aetron Photo Documentation, *Ziziphus mauritiana* Lam.

INTRODUCTION

Herbal medicines have a long therapeutic history. But the quality control and quality assurance remains a challenge because of the high variability of chemical components involved. Herbal drugs, singularly and in combinations, contain a myriad of compounds in

complex matrices in which no single active constituent is responsible for the overall efficacy.[1] This creates a challenge in establishing quality control standards for raw materials and standardization of finished herbal drugs. The therapeutic effects of herbal medicines are based on the complex interaction of numerous ingredients in combination, which are totally different from those of chemical drugs.[2] Many kinds of fingerprint analysis methods to control the quality of herbal drugs have gradually come into being, such as thin layer chromatography, gas chromatography, high performance liquid chromatography etc., Chromatographic fingerprint analysis of herbal drugs represents a comprehensive qualitative approach for species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their related products.[3]

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, etc., enable it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses.[4]

HPTLC can be used to separate mixtures of compounds for either analytical identification or quantification, or for preparative purification.[5] A mixture of compounds is dissolved in liquid and the solution (mobile phase) is transported along an immobile non-soluble adsorbent (stationary phase). Along the way, the solute molecules interact with the stationary phase in repetitive adsorption/desorption steps. Various compounds have different degrees of interaction with the adsorbent and therefore have different mobilities. A compound that has stronger interactions with the stationary phase will move along slower than a compound that has weaker interaction with the adsorbent. This leads to separation due to difference in mobility.[6]

MATERIALS AND METHODS

The selected fruit under study was collected and authenticated. It was dried, powdered and stored. The crude extracts were extracted using selective solvents through standard

procedures. The crude extracts were optimized for selection of solvent, amount of solvent and time of extraction.

The solvents used were of analytical grade quality. Distilled water used for the analysis was purified with Sartorius water purification unit. Standard volumetric flasks, pipettes of class A grade were used throughout the experiment. All the solvents were filtered through 0.45µ Millipore filter membrane and degassed in an ultra sonic bath.

1) Instrumentation

- CAMAG HPTLC
- CAMAG Linomat IV as applicator.
- CAMAG Scanner III (Densitometry)
- Deuterium, Mercury and Tungsten as source of radiation.
- 100µL syringe for sample loading.
- winCATS 1.4.2 software system.
- Aetron Photo Documentation Unit.

2) Sample Preparation

A *Ziziphus mauritiana* Lam. (fruit) extract

Accurately weighed 1g of the dried fruit powder was placed in four stoppered test tubes and 10cm³ of methanol was added in each of the test tubes. Four different sample preparations were prepared as follows:

A 1: *Ziziphus mauritiana* powder 1g + 10cm³ of methanol + 1drop of ammonia.

A 2: *Ziziphus mauritiana* powder 1g + 10cm³ of methanol + I drop of acetic acid.

A 3: *Ziziphus mauritiana* powder 1g + 10cm³ of methanol+ 1 drop each of ammonia and acetic acid.

A 4*:* *Ziziphus mauritiana* powder 1g + 10cm³ of methanol

The test tubes were stoppered and sealed and kept on rotatory shaker for 3 hrs. The contents of the tube after extraction were filtered through Whatmann filter paper No. 41. The extracts collected were further filtered through 0.45µ Millipore filter membrane.

3) Chromatography

Chromatography was performed on silica gel 60 F_{254} HPTLC pre-coated plates. Samples of definite volume in microlitres was loaded 10mm from the edge of the plates by means of

Camag Linomat IV applicator and the plates were developed to a distance of 80mm in a Camag twin-trough chamber previously equilibrated with the respective mobile phase.

Chromatograms were evaluated densitrometrically at definite wavelengths using Camag Scanner III with a computer system in conjunction with winCATS 1.4.2 Version Software. Chromatographic conditions for establishing the HPTLC fingerprint of *Ziziphus mauritiana* Lam. (fruit) extract is given in Table 1.0.

Table 1.0: Optimized conditions for HPTLC of fruit extract of *Ziziphus mauritiana* Lam.

PARAMETERS	DESCRIPTION
Instrument	CAMAG HPTLC
Stationary Phase	Silica gel 60 F_{254} HPTLC pre-coated plates
Spotting mode	CAMAG LINOMAT IV Sample Applicator
Band width	8.0mm
Spotting volume	10µL
Syringe	CAMAG Linomat Syringe 695.0014
Mobile phase	Methyl alcohol: Toluene: Ethyl acetate (4.0: 6.0:1) v/v/v
Volume of mobile phase	11.0cm³
Development mode	CAMAG Twin Trough Chamber
Development Distance	8.0cm
Chamber saturation time	30mins.using Whatmann filter paper no 41
Wavelength	254nm,366nm and visible
Densitometric scanner	CAMAG TLC SCANNER III
Software	winCATS version1.4.2
Radiation source	Deuterium, Mercury and Tungsten

RESULTS AND DISCUSSION

Baseline Peak display of *Ziziphus mauritiana Lam.* (fruit) at 366nm (sample A2).

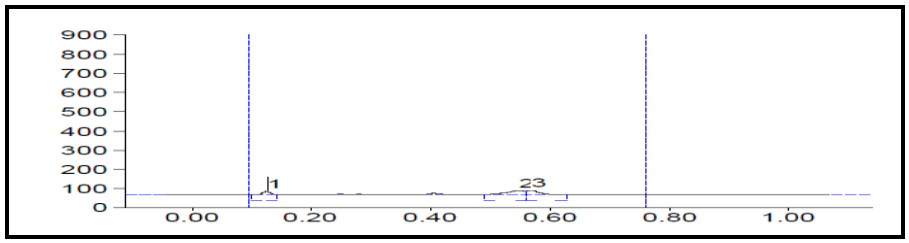


Fig 1.1

The different peaks with their retention factor (R_f) and respective areas are as shown in Table 1.1

Peak No.	R_f in cm	Area under curve (AUC) in AU
1	**0.13**	**213.5**
2	**0.55**	**604.5**
3	**0.57**	**427.3**

Baseline Peak display of *Ziziphus mauritiana Lam.* (fruit) at 366nm after Derivatization with 5% Sulfuric acid.

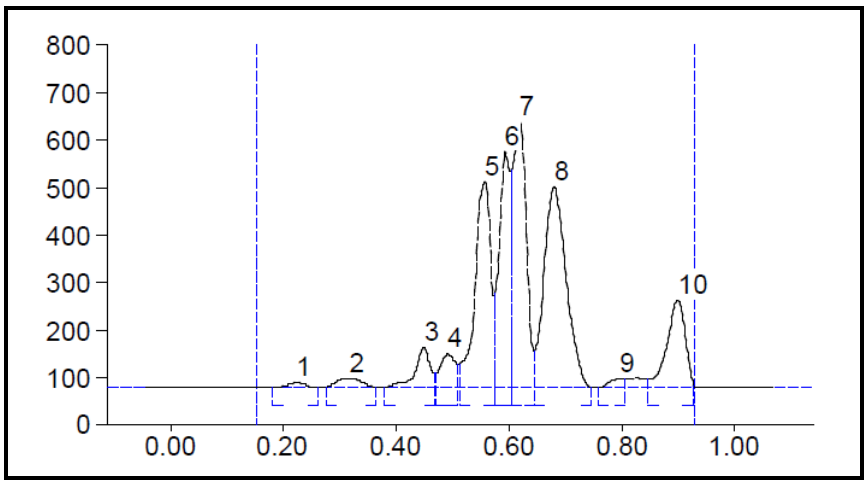


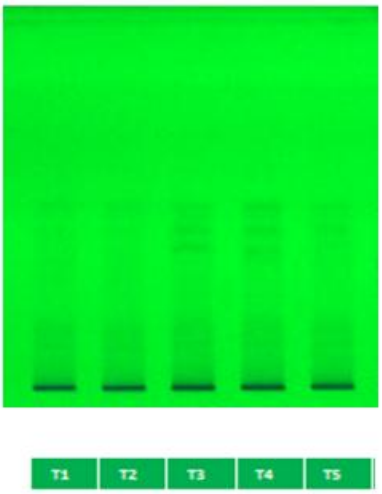
Fig 1.3

The different peaks with their retention factor (R_f) and respective areas are shown in Table 1.3

Peak No.	R_f in cm	Area under curve (AUC) in AU
1	0.22	316.1
2	0.32	798.2
3	0.45	1964.8
4	0.49	1675.3
5	0.56	10361.6
6	0.60	8214.9
7	0.62	113171.1
8	0.68	14087.5
9	0.80	428.8
10	0.90	5061.4

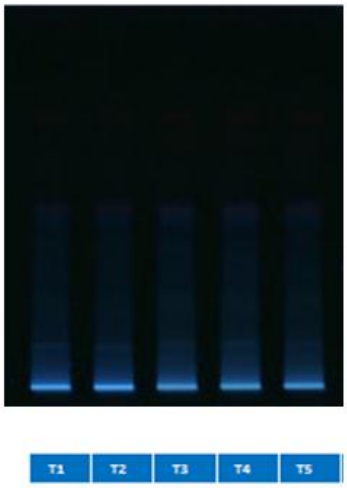
The chromatographic fingerprint for methanolic extract of *Ziziphus mauritiana* Lam. plus one drop acetic acid showed ten different components having maximum absorbance as seen in the chromatogram (Fig 1.3).

HPTLC Photo plates at 254nm and 366nm are shown in Plate 1.0.



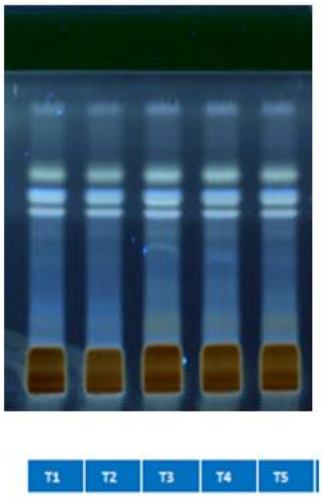
(Fig 1.5)

254 nm



(Fig 1.6)

366 nm



(Fig 1.7)

366 nm after derivatization

T1	T2	T3	T4	T5
A1	A1	A2	A3	A4

SAMPLE A – *Ziziphus mauritiana* (Tracks T1 to T5).

HPTLC is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images. Complex information about the entire sample is available at a glance. Multiple samples are seen simultaneously, so that reference and test samples can be compared for identification. HPTLC fingerprint is also suitable for rapid and simple authentication and comparison of the subtle difference among samples with identical plant resource but different geographic locations and hence is a very important tool in herbal drug industry.

Baseline Peak display of *Ziziphus mauritiana Lam.* (fruit) at 366nm showed three peaks with their retention factor (R_f) between 0.13 and 0.57. After derivatization with 5% Sulfuric acid, the chromatogram showed ten different well resolved peaks representing ten different components having maximum absorbance as seen in the chromatogram (Fig 1.3).

CONCLUSION

The HPTLC methods demonstrated are simple, economical and reliable. The fingerprints established are representative of a given species. These methods are suitable for monitoring the identity and purity of crude drugs and for detecting adulteration. Fingerprinting of *Ziziphus mauritiana* Lam. can be helpful in the herbal industry for quality control of herbal medicines and herbal preparations thus enabling it to be a powerful analytical tool for chromatographic information of complex mixtures of inorganic organic and biomolecules.

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