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# Pharmacognostic evaluation and HPTLC fingerprinting of *Nicotiana tabacum* leaf collected from different geographical regions of India

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## ABSTRACT

*Nicotiana tabacum, a traditional medicinal plant which is valued for its benefits as sedative, laxative, tonic , emetic, carminative, antispasmodic and vermifuge, and in management of skin diseases, local infections, bronchitis, asthma and inflammation. The leaves has been employed in curing old ulcers and painful tumors and for the extraction of the active principle i.e. nicotine which, usually in the form of sulphate, is widely used as an insecticide and in the production of synthetic nicotinic acid and nicotinamide. The current study was therefore carried out to provide requisite pharmacognostic details about the leaves of Nicotiana tabacum. Pharmacognostic evaluation included examination of morphological and microscopical characters; physicochemical properties, phytochemical analysis, and HPTLC fingerprint. The powder microscopy showed the presence of glandular trichome (single celled head and multicellular stalk), covering trichome (multicellular unisereate covering trichome), paracytic stomata, acicular and prismatic calcium oxalate crystals and spiral vessel with bordered thick wings. The phytochemical screening revealed the presence of alkaloids, flavonoids, phytosterols, triterpinoids, tannins and carbohydrates. The Rf values detected at 400 nm by qualitative densitometric HPTLC fingerprint, can be used as identifying marker for petroleum ether extract. The present study will provide the information with respect to identification and authentication of crude drug.*

**Key words:** *Nicotiana tabacum*, leaves, pharmacognosy, HPTLC fingerprint.

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## INTRODUCTION

Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the

plant material may be useful for proper standardization of herbals and its formulations [1]. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [2]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time [3]. The plant *Nicotiana tabacum* belonging to family Solanaceae is a stout, viscid annual herb upto 1-3 m in height and is cultivated through out India. It is known as Tamaku in Hindi and Hogesoppu in Kannada [4]. Traditionally it has been used to treat skin diseases, local infections, bronchitis, asthma and inflammation [5]. An ointment made by simmering the leaves in lard has been employed in curing old ulcers and painful tumors [6]. The plant leaves are also utilized for the extraction of the active principle i.e. nicotine which, usually in the form of sulphate, is widely used as an insecticide. Nicotine is also used in the production of synthetic nicotinic acid and nicotinamide [7]. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of leaves; physicochemical parameters like ash values, extractive values, moisture content etc. has been evaluated for different samples of leaf collected from different geographical regions and preliminary phytochemical analysis of different samples of *Nicotiana tabacum* leaf has been done to identify the chemical constituents and HPTLC fingerprinting has been performed which may be used as markers for quality evaluation, and standardization of this drug.

## MATERIALS AND METHODS

### Plant material

The *Nicotiana tabacum* plants were collected from the Jhajjar distt. (Haryana), Shimoga distt. (Karnataka), Ayodhya distt. (U.P) and Satara distt. (Maharashtra). These were subsequently analysed and authenticated at Department of Botany, D.V.S College of Art & Science, Shimoga, Karnataka and Department of Botany, Faculty of Science, Jamia Hamdard University, New Delhi.

### Preparation of Plant material

The leaves of *Nicotiana tabacum* were separated for each plant sample from different geographical region and then washed with water, dried at normal room temperature, powdered through grinder to make a coarse powder and stored in air tight containers respectively. The macroscopy and microscopy of the leaf were studied according to the method described by Brain and Turner [8]. Physicochemical parameters were calculated according to the methods described by Mukherjee [9]. Preliminary phytochemical analysis of powdered leaf was performed as described by Khandelwal [10] and Kokate [11].

### Sample preparation for HPTLC fingerprinting

All dried powdered samples were sonicated with 25 ml of petroleum ether separately for thirty minutes. The petroleum ether extracts thus obtained were evaporated to dryness in china dish on water bath to get the residue. Each extract residue was re dissolved in 1ml of chromatographic grade solvent i.e. petroleum ether, which were then used for sample application on pre-coated silica gel $60F_{254}$ aluminium sheets.

**Optimization of HPTLC solvent system**
A number of solvent systems were tried for different extracts, but the most satisfactory resolution was obtained in the solvent n-Hexane: ethyl acetate (5:1)

**Sample application-** Application of bands of each sample extract was carried out (4mm in length) and a concentration of 1µl for leaf was applied using spray technique. Sample were applied in duplicate on pre-coated silica gel $60F_{254}$ aluminium sheets with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

**Development of chromatogram-** After the application of spots, the chromatogram was developed in Twin trough glass chamber 20x 10 cm saturated with solvent n Hexane: ethyl acetate (5:1) for 15 min.
**Detection of spots-** The air-dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 400nm after treatment with anisaldehyde sulfuric acid reagent. The R$f$ values and finger print data were recorded by WIN CATS software. The 3D display of all tracks and fingerprints of all different samples at 400nm for petroleum ether extract.

## RESULTS AND DISCUSSION

**Macroscopic Characters of the leaf**
The leaf of *Nicotiana tabacum* is green in colour, odour is characteristic of nicotine, taste is bitter, 60-100cm in length, 35-45cm in width and ovate, elliptic or lanceolate in shape. Acuminate at the apex, usually sessile or some time petiolate, margin is entire with reticulate venation as shown in fig.1 (a, b).

**Microscopic characters of the leaf**
**Transverse section of Leaf:** It is a dorsiventral leaf. In transverse section of midrib and lamina following tissues were observed

**Midrib:** In the epidermal layer, lamina is continuous with the midrib region. Vascular bundles are small, single and less prominent, arc shaped and is present more towards ventral surface and consist of 3-5 rows of lignified xylem and thin arc of non lignified phloem. Surface preparations show paracytic type of stomata.

**Lamina:** The upper epidermis shows the presence of single layered, rectangular cells, distinct cuticle, multicellular unisereate covering trichome and glandular trichome with single celled head and multicellular stalk. Few stomata (paracytic stomata) are also seen. In the mesophyll, the palisade constitutes single layered, compact and radially enlonged cells while the spongy parenchyma consist of four to six layered cells. Lower epidermis is some what similar to upper epidermis, has many stomata and numerous covering and glandular trichomes. The results are shown in fig 2 (a, b, c, d)

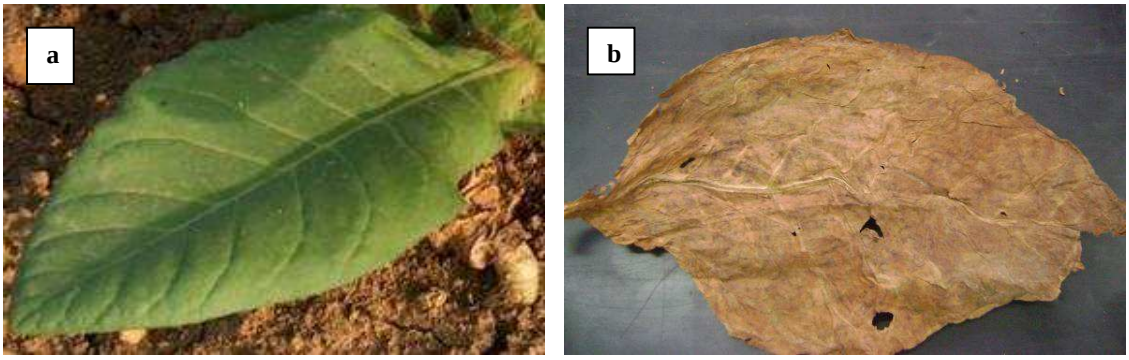



**Fig: 1 (a) *Nicotiana tabacum* leaf fresh (b) *Nicotiana tabacum* leaf dry**

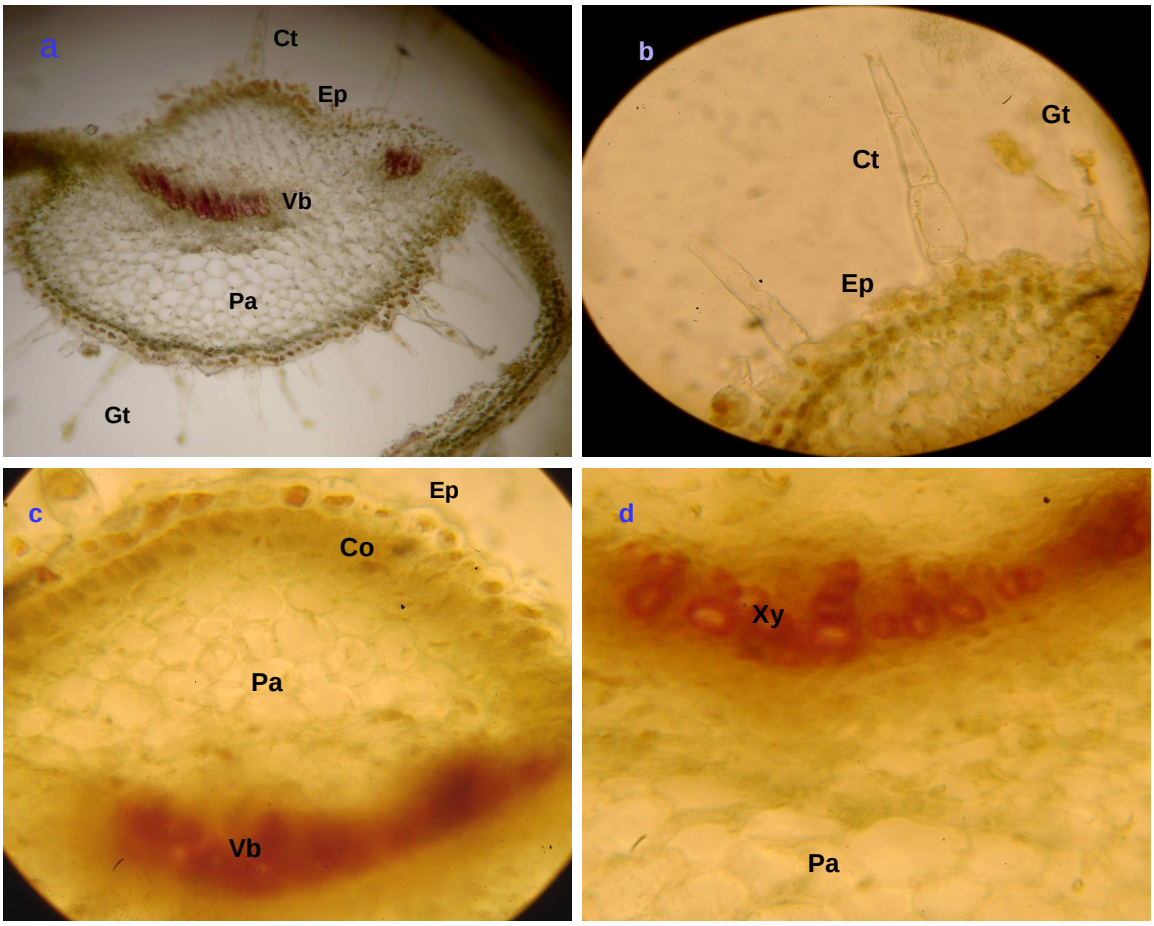



**Fig: 2. Intact Microscopy of *Nicotiana tabacum* leaf (a) T.S of *nicotiana tabacum* leaf (b) T.S showing covering trichome, glandular trichome and epidermis (c) T.S showing epidermis, collenchyma, parenchyma and vascular bundle (d) T.S showing xylem and lower parenchyma. Ep-epidermis, Ct- covering trichome, Gt-glandular trichome, Pa-parenchyma,Co-collenchyma, Vb-vascular bundles, Xy-xylem.**

## Powder microscopy

Powder microscopy of *Nicotiana tabacum* leaf revealed following characters: Glandular trichome (single celled head and multicellular stalk), Covering trichome (multicellular unisereate

covering trichome), Paracytic stomata, Acicular and prismatic calcium oxalate crystals and Spiral vessel with bordered thick wings. The results are shown in fig 3 (a, b, c, d, e, f)

**Preliminary Phytochemical Screening**

For the phytochemical studies, the preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extract for *Nicotiana tabacum* leaf have been done. The result revealed the presence of alkaloids, flavonoids, phytosterols, triterpinoids, tannins and carbohydrates. The results are shown in table 1

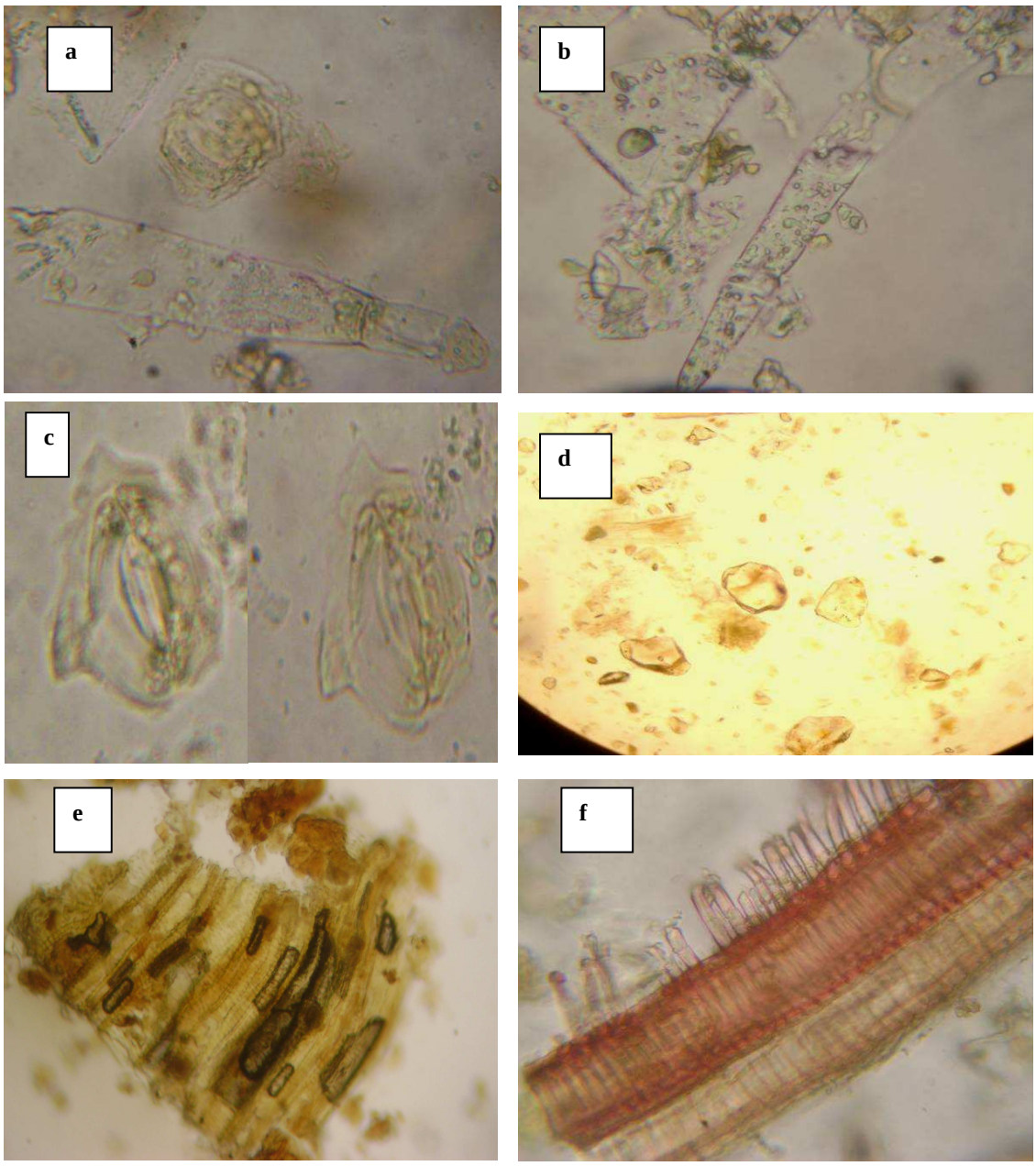



**Fig: 3. Powder microscopy of *Nicotiana tabacum* leaf (a) glandular trichome (b) covering trichome (c) Paracytic stomata (d) calcium oxalate crystals (e) spiral vessel (f) spiral vessel with bordered thick wings**

## Physicochemical parameters

Significant amount of acid insoluble ash has been detected which indicates presence of various silicacious substances. Cellulosic substances also contributed significantly in total ash as indicated by water soluble ash. Less amount of non-polar substance in comparison to polar one were found as these did not showed much percentage yield of ether soluble extractives. However, alcoholic and aqueous extractives showed significant yields. Significant amount of moisture have also been found in air dried materials of *Nicotiana tabacum.* Foreign organic matter was also calculated which is useful tool in detection of contaminant which may be sand, soil, stone, dust or animal excreta etc. The plant material should be free from these contaminants. Comparison of physicochemical parameter such as moisture content, ash value, extractive value provide a useful information to distinguish the plant from other plants. The results are shown in table 2, 3, 4, 5.

**Table: 1. Phytochemical screening of leaf extracts of *Nicotiana tabacum***

| Chemical Constituent | Tests | Pt. ether Ext. | $CHCl_3$ Ext. | EtOH Ext. | Aq. Ext. |
|---|---|---|---|---|---|
| Alkaloids | Dragendroff's test | -ve | +ve | +ve | -ve |
| | Hagers | -ve | +ve | +ve | -ve |
| | Mayer's test | -ve | -ve | +ve | -ve |
| Carbohydrates | Molisch's test | -ve | -ve | +ve | +ve |
| | Benedicts test | -ve | -ve | +ve | +ve |
| | Fehling's test | -ve | -ve | +ve | +ve |
| Saponins | Foam test | -ve | -ve | -ve | -ve |
| Phenols | Lead acetate test | -ve | -ve | +ve | +ve |
| | FerricChloride test | -ve | -ve | +ve | +ve |
| Flavonoids | Shinoda test | -ve | -ve | +ve | +ve |
| Acid compound | Sodium bicarbonate test | -ve | -ve | +ve | +ve |
| Tannins | Gelatin test | -ve | -ve | +ve | +ve |
| Phytosterols | Salkowski test | +ve | +ve | -ve | -ve |
| | Libermann Burchard test | +ve | +ve | -ve | -ve |
| Triterpenes | Salkowski test | +ve | +ve | -ve | -ve |
| | Libermann's test | +ve | +ve | -ve | -ve |

*+ve posiive (present), -ve negative (absent)*

**Table: 2. Extractive value for successive extraction of solvent in *Nicotiana tabacum* leaf of different geographical region**

| Region of leaf sample | Solvent | Extractive values (% w/w) |
|---|---|---|
| **Haryana** | Pet. Ether | 4.86±0.08 |
| | Chloroform | 1.85±0.09 |
| | Ethanol | 19.42±0.48 |
| | Water | 15.72±0.65 |
| **Karnataka** | Pet. Ether | 5.05±0.05 |
| | Chloroform | 2.27±0.29 |
| | Ethanol | 18.87±0.10 |
| | Water | 20.46±0.51 |
| **Maharashtra** | Pet. Ether | 3.08±0.08 |
| | Chloroform | 1.86±0.09 |
| | Ethanol | 16.11±0.62 |
| | Water | 18.46±0.75 |
| **Uttar Pradesh** | Pet. Ether | 3.82±0.18 |
| | Chloroform | 1.08±0.12 |
| | Ethanol | 22.36±0.8 |
| | Water | 12.14±0.31 |

*Values are in mean± Standard deviation, where n=3.*

**Table: 3. Ash value determination of *Nicotiana tabacum* leaf of different geographical region**

| Region of leaf sample | Ash parameter | Ash values(% w/w) |
|---|---|---|
| **Haryana** | Total ash | 22.76±0.058 |
| | Water soluble ash | 14.51±0.017 |
| | Acid insoluble ash | 1.39±0.065 |
| **Karnataka** | Total ash | 22.19±0.610 |
| | Water soluble ash | 19.89±0.241 |
| | Acid insoluble ash | 2.35±0.469 |
| **Maharashtra** | Total ash | 26.51±0.17 |
| | Water soluble ash | 19.38±0.17 |
| | Acid insoluble ash | 2.23±0.04 |
| **Uttar Pradesh** | Total ash | 19±0.41 |
| | Water soluble ash | 18.015±0.27 |
| | Acid insoluble ash | 4.75±0.16 |

*Values are in mean± Standard deviation, where n=3.*

**Table: 4. foreign organic matter in *Nicotiana tabacum* leaf**

| Region of leaf sample | % foreign organic matter |
|---|---|
| **Haryana** | 5.83±0.46 |
| **Karnataka** | 1.78±0.38 |
| **Maharashtra** | 2.49±0.19 |
| **Uttar Pradesh** | 1.73±0.16 |

*Values are in mean± Standard deviation, where n=3.*

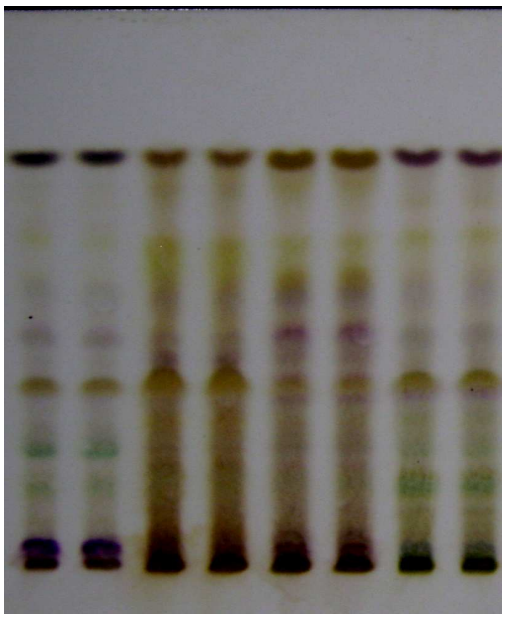
**Table: 5. Loss on drying for *Nicotiana tabacum* leaf samples**

| Region of leaf sample | % LOD |
|---|---|
| **Haryana** | 11.149±0.18 |
| **Karnataka** | 10.124±0.32 |
| **Maharashtra** | 11.23±0.14 |
| **Uttar Pradesh** | 12.25±0.27 |

*Values are in mean± Standard deviation, where n=3.*

**Table: 6. fingerprint data of each sample petroleum ether extract (at 400 nm) sprayed.**
*(peaks having Rf value < 1 are omitted)*

| Sample No | Region | Number of peaks | Corresponding Rf values |
|---|---|---|---|
| 1 | Karnataka (leaf) | 12 | 0.1, 0.18, 0.26, 0.42, 0.48, 0.53, 0.57, 0.66, 0.76, 0.84, 0.88, 0.94 |
| 2 | Maharashtra(leaf) | 9 | 0.19, 0.23, 0.33, 0.43, 0.53, 0.57, 0.7, 0.72, 0.94 |
| 3 | U.P(leaf) | 9 | 0.13, 0.2, 0.28, 0.33, 0.42, 0.55, 0.66, 0.75, 0.94 |
| 4 | Haryana(leaf) | 11 | 0.1, 0.13, 0.18, 0.26, 0.35, 0.44, 0.54, 0.68, 0.76, 0.85, 0.94 |



**KR MH UP HR**
**(Leaves samples)**

**Fig: 4. HPTLC chromatogram of all drugs samples of *Nicotiana tabacum* at 400nm in petroleum ether extracts (anisaldehyde sulphuric acid sprayed data).**
*KR (Karnataka leaf), MH (Maharashtra leaf), UP (Uttarpradesh leaf), HR (Haryana leaf). Samples applied in duplicate.*

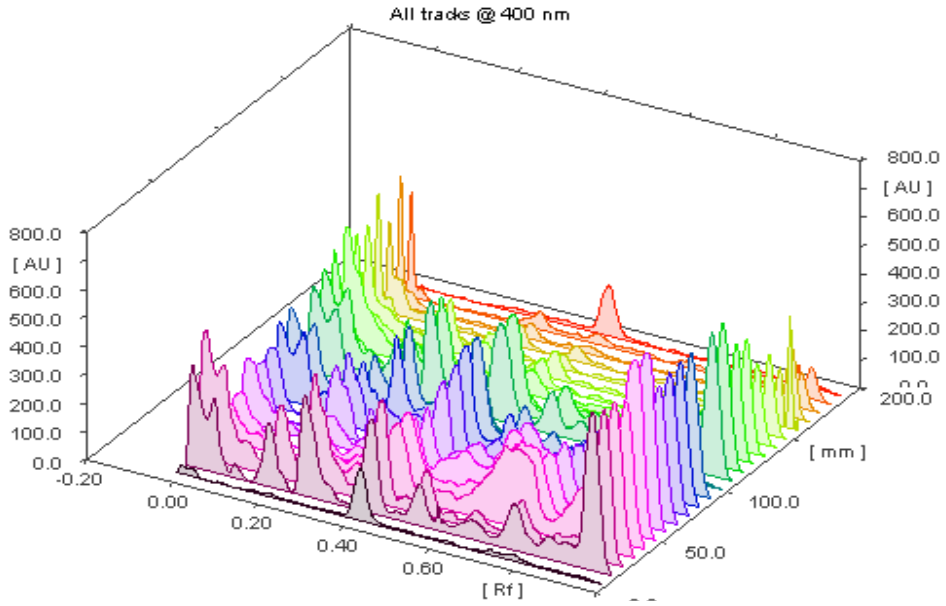



**Fig: 5. 3D display of all samples at 400 nm.**

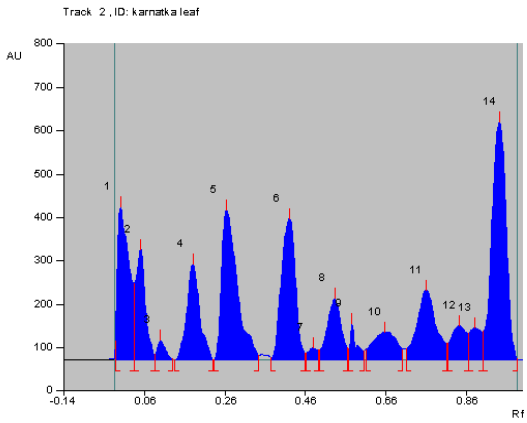



**Fig: 6. HPTLC fingerprinting of Karnataka leaf**

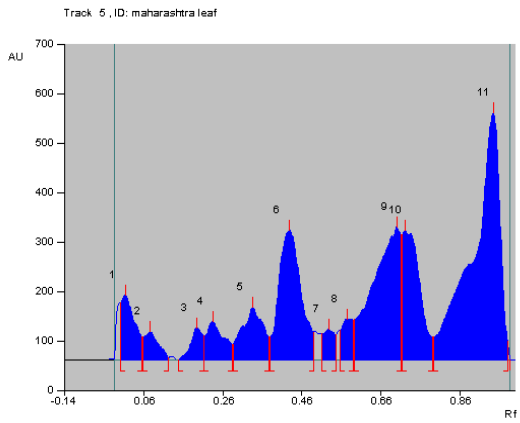



**Fig: 7. HPTLC fingerprinting of Maharashtra leaf**

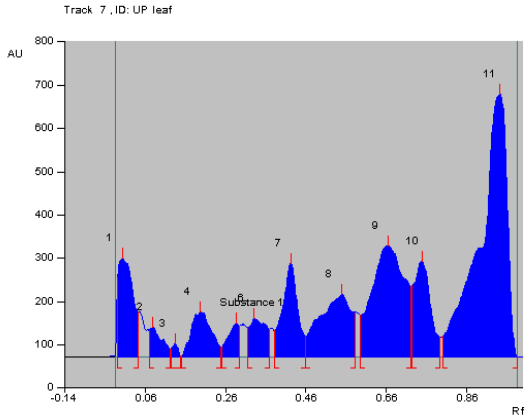



**Fig: 8. HPTLC fingerprinting of U P leaf**

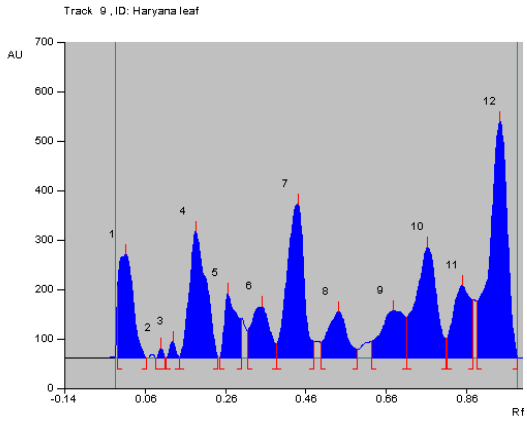



**Fig: 9. HPTLC fingerprinting of Haryana leaf**

## HPTLC fingerprinting of leaf

HPTLC fingerprinting was carried out for all leaf samples collected from different geographical regions in petroleum ether extract after spraying with anisaldehyde sulphuric acid reagent using CAMAG HPTLC system, so many phytochemical variations were observed. The petroleum ether extract of karnataka leaf sample at 400nm showed the presence of highest no of compounds and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. 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. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved. Though further work to characterize the other chemical constituents and quantitative estimation with marker compounds is also necessary, these data can also be considered along with the other values for fixing standards to this plant. The results for HPTLC fingerprinting are shown in fig. 4, 5, 6, 7, 8, 9 and table 6.

## CONCLUSION

Thus the organoleptic, microscopic characters, physico-chemcial study, preliminary phytochemical screening and HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. The adulterants if any in this plant material can be easily identified by using these results.

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