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Der Pharmacia Sinica, 2011, 2 (5): 1-11

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Pharmacognostic evaluation and HPTLC fingerprinting of *Nicotiana Tabacum* stem collected from different geographical regions of India

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ABSTRACT

Nicotiana tabacum, plant has been used traditionally 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 current study was therefore carried out to provide requisite pharmacognostic details about the stem 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 Scalariform vessels, Group of fibers, Parenchymatous cells, Spiral xylem vessel, Brownish matter and Phloem fiber. The phytochemical screening revealed the presence of alkaloids, flavonoids, phytosterols, triterpinoids, and tannins. 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.

Keywords: *Nicotiana tabacum*, pharmacognostic evaluation, phytochemical screening, HPTLC fingerprint, alkaloids.

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. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [1-2]. 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, Tamrakuta in Sanskrit and Hogesoppu in Kannada. Traditionally it has been used to treat skin diseases, local infections, bronchitis, asthma and inflammation. The plant is 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. The objective of the present study is to evaluate various pharmacognostic standards like macroscopy and microscopy of stem; physicochemical parameters like ash values, extractive values, moisture content etc. has been evaluated for different samples of stem collected from different geographical regions and preliminary phytochemical analysis of different samples of *Nicotiana tabacum* stem 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 [4-7].

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 verified by 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 stems 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 of Brain and Turner [8]. Physicochemical parameters were calculated according to the methods described by Mukherjee, Prabhu and Lobo [9-11]. Preliminary phytochemical analysis of powdered stem sample was performed according to methods described by Khandelwal [12] and Kokate [13].

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 5µl for stem 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 data of 3D display of all tracks and fingerprints of all different stem samples at 400nm for petroleum ether extract was obtained.

RESULTS AND DISCUSSION

Macroscopic Characters of the stem
The stem of *Nicotiana tabacum* is Yellowish brown to dark brown in color, odor is faint to characteristic, taste is bitter, up to 2m or more in length and 0.5-5cm or more in width and shape is cylindrical, rough surface and with fibrous fracture [Figure1].

Microscopic characters of the stem
Transverse section of stem is almost circular in shape. Epidermis consists of single row of flattened closely arranged cells surrounded by cuticle. Many glandular and covering trichomes are present. In cortex region three distinct portions can be clearly observed. The outermost 3-4 layered collenchymatous cells are closely arranged. It is followed with 4-5 layers of loosely arranged parenchymatous cells with intercellular space. The cells are more or less spherical in shape. The inner most layer of cortex i.e. endodermis is made up of closely arranged, radially elongated parenchymatous cells. Vascular bundles were collateral and conjoint arranged in form of ring. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Xylem consists of tracheids, fibres and few vessels. Pith region is large and made up of thin walled, polygonal parenchymatous cells with intracellular spaces [Figure 2; a, b, c, d, e and f].

Powder microscopy
Powder microscopy of *Nicotiana tabacum* stem revealed following characters: Scalariform vessels, Group of fibers, Parenchymatous cells, Spiral xylem vessel, Brownish matter, and Phloem fiber [Figure 3; a, b, c, d, e, and f].

Preliminary Phytochemical Screening
For the phytochemical studies, the preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extract for *Nicotiana tabacum* stem have been done. The result revealed the presence of alkaloids, flavonoids, phytosterols, triterpinoids, tannins and carbohydrates [Table 1].

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 [Table 2, 3, 4 and 5].

Figure 1. *Nicotiana tabacum* stem

HPTLC fingerprinting

HPTLC fingerprinting was carried out for all stem 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 and Uttar Pradesh stem sample at 400nm showed the presence of highest no of compounds (13 each) and purity of the sample was confirmed by comparing the absorption spectra at start, middle and end position of the band [Figure 4, 5, 6, 7, 8, 9] and [Table 6].

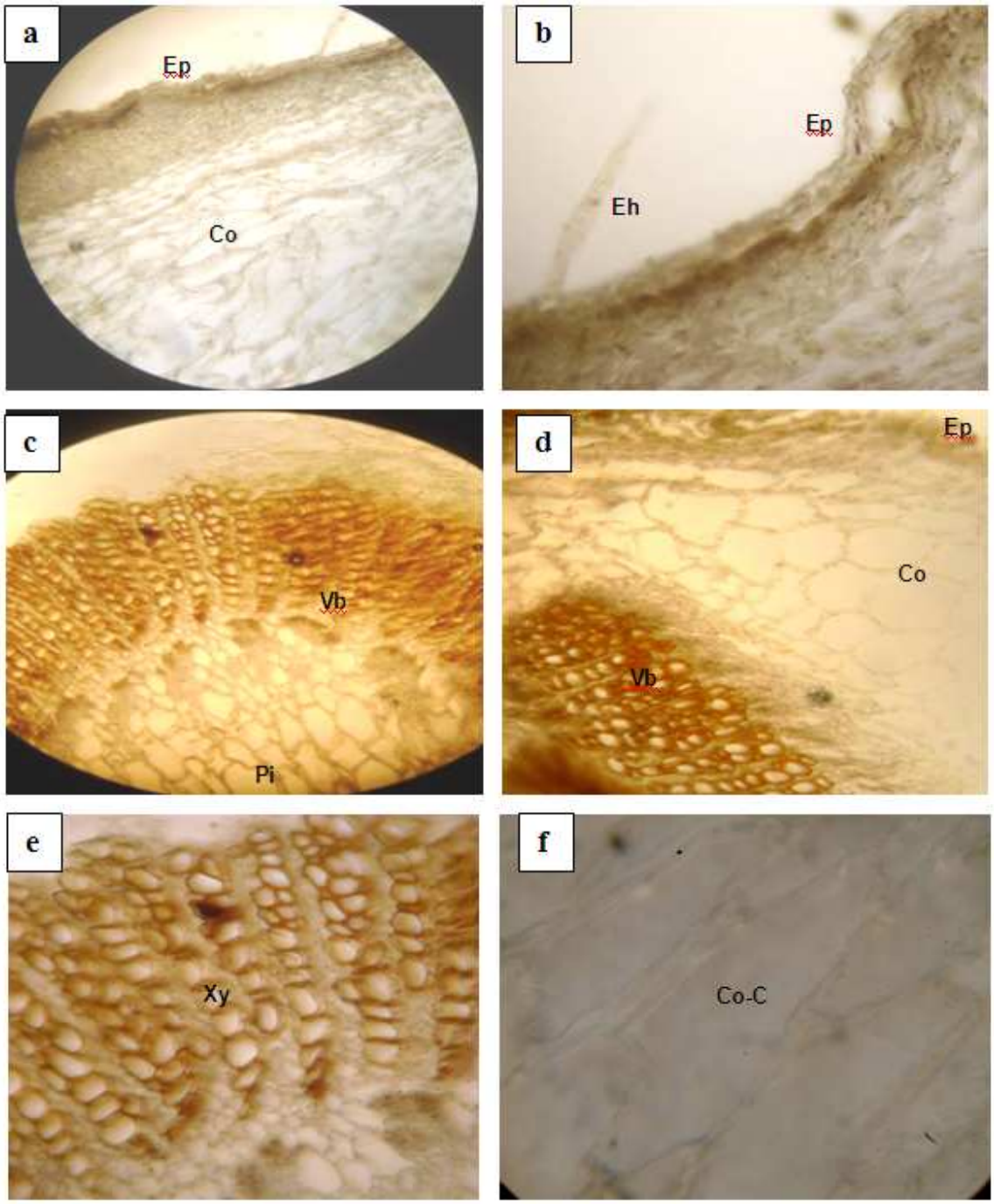

Figure 2. Intact Microscopy of *Nicotiana tabacum* stem (a) T.S showing epidermis and cortex (b) T.S enlarged showing epidermis and epidermal hair (c) T.S showing vascular bundle and pith (d) T.S showing epidermis, cortex and vascular bundle (e) T.S showing xylem vessel (f) T.S enlarged showing cortex cells. Ep-epidermis, Eh- epidermal hair, Co-cortex, Co-C-cortex cell, Vb-vascular bundles, Xy-xylem, Ph-phloem, Pf-pericyclic fibre, Pi-pith.

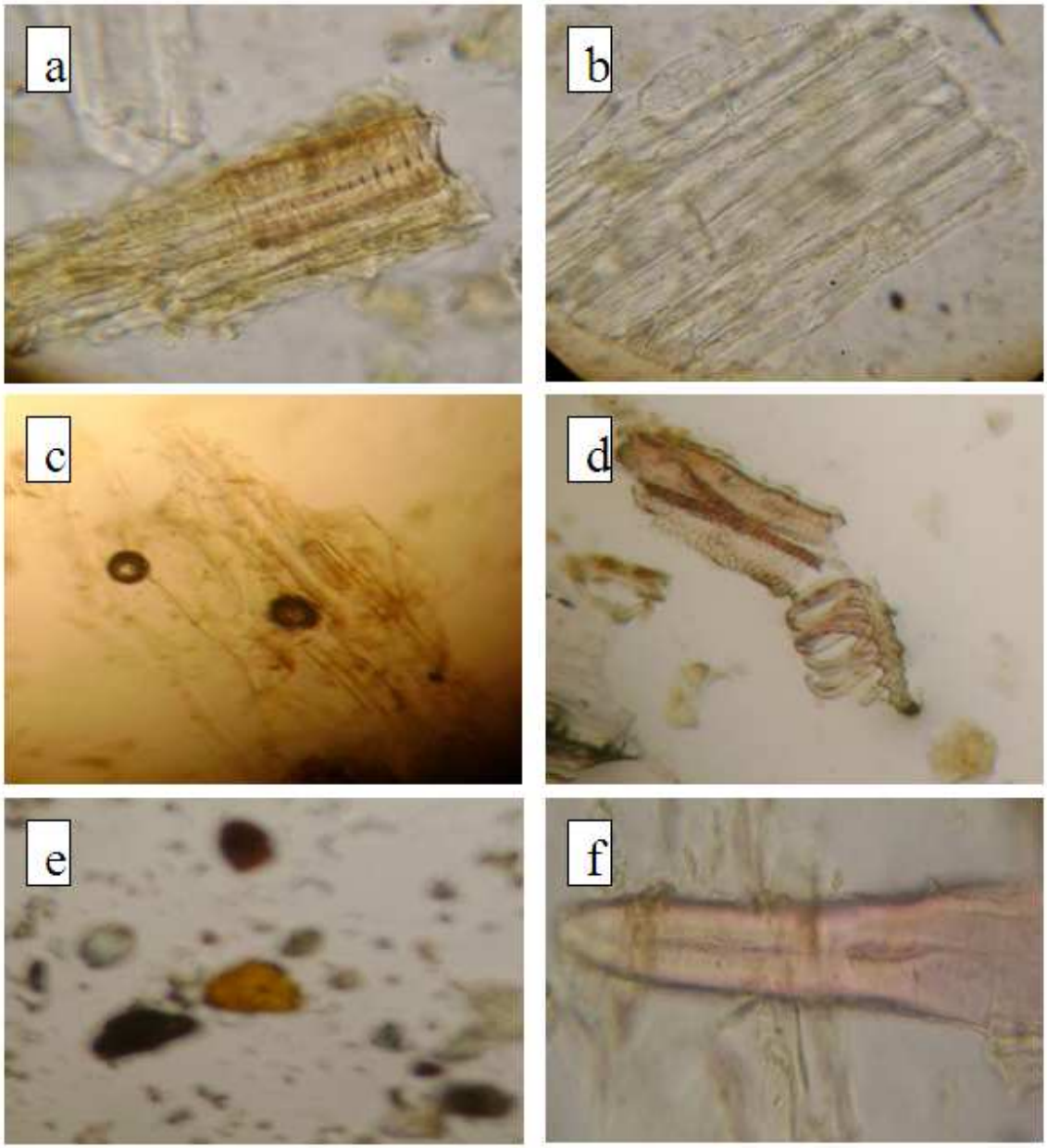

Figure 3. Powder microscopy of *Nicotiana tabacum* stem (a) scalariform vessels (b) group of fibers (c) Parenchymatous cells (d) spiral xylem vessel (e) brownish matter (f) Phloem fiber

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.

Table 1. Phytochemical screening of stem extracts of *Nicotiana tabacum*

Chemical Constituent	Tests	Pt. ether Ext.	$CHCl_3$ Ext.	EtOH Ext.	Aqu. 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* stem of different geographical region

Region of stem sample	Solvent	Extractive values (% w/w)
Haryana	Pet. Ether	0.48±0.052
	Chloroform	0.35±0.05
	Ethanol	9.68±0.07
	Water	11.37±0.45
Karnataka	Pet. Ether	1.13±0.05
	Chloroform	0.77±0.03
	Ethanol	11.60±0.11
	Water	13.18±0.56
Maharashtra	Pet. Ether	0.76±0.03
	Chloroform	0.45±0.052
	Ethanol	11.36±0.40
	Water	8.73±0.16
Uttar Pradesh	Pet. Ether	1.03±0.017
	Chloroform	0.67±0.03
	Ethanol	13.45±0.10
	Water	15.90±0.09

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

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

Region of stem sample	Ash parameter	Ash values(% w/w)
Haryana	Total ash	1.06±0.07
	Water soluble ash	11.1±0.06
	Acid insoluble ash	14.95±0.02
Karnataka	Total ash	2.57±0.11
	Water soluble ash	13.96±0.15
	Acid insoluble ash	12.95±0.084
Maharashtra	Total ash	1.19±0.04
	Water soluble ash	13.27±0.102
	Acid insoluble ash	10.28±0.014
Uttar Pradesh	Total ash	3.33±0.098
	Water soluble ash	15.9±0.21
	Acid insoluble ash	16.21±0.12

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

Table 4. foreign organic matter in *Nicotiana tabacum* stem

Region of stem sample	% foreign organic matter
Haryana	2.44±0.41
Karnataka	0.93±0.13
Maharashtra	1.31±0.11
Uttar Pradesh	0.48±0.06

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

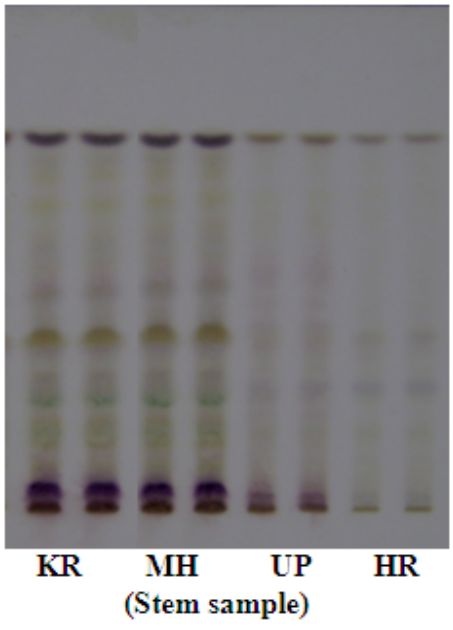

Figure 4. HPTLC chromatogram of all drugs samples of *Nicotiana tabacum* at 400nm in petroleum ether extracts (anisaldehyde sulphuric acid sprayed data).
KR Karnataka stem), MH (Maharashtra stem), UP (Uttarpradesh stem), HR (Haryana stem). Samples applied in duplicate.

Table 5. Loss on drying for *Nicotiana tabacum* stem samples

Region of stem sample	% LOD
Haryana	8.46
Karnataka	7.36
Maharashtra	7.67
Uttar Pradesh	7.56

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 R*f* value < 1 are omitted)

Sample No	Region	Number of peaks	Corresponding Rf values
1	Karnataka(stem)	13	0.1, 0.16, 0.19, 0.28, 0.34, 0.44, 0.55, 0.62, 0.69, 0.77, 0.85, 0.88, 0.94
2	Maharashtra(stem)	12	0.1, 0.2, 0.27, 0.34, 0.44, 0.56, 0.63, 0.71, 0.78, 0.85, 0.89, 0.94
3	U.P(stem)	13	0.2, 0.31, 0.35, 0.4, 0.44, 0.52, 0.6, 0.64, 0.67, 0.74, 0.8, 0.87, 0.94
4	Haryana(stem)	10	0.19, 0.31, 0.4, 0.44, 0.5, 0.56, 0.58, 0.8, 0.87, 0.94

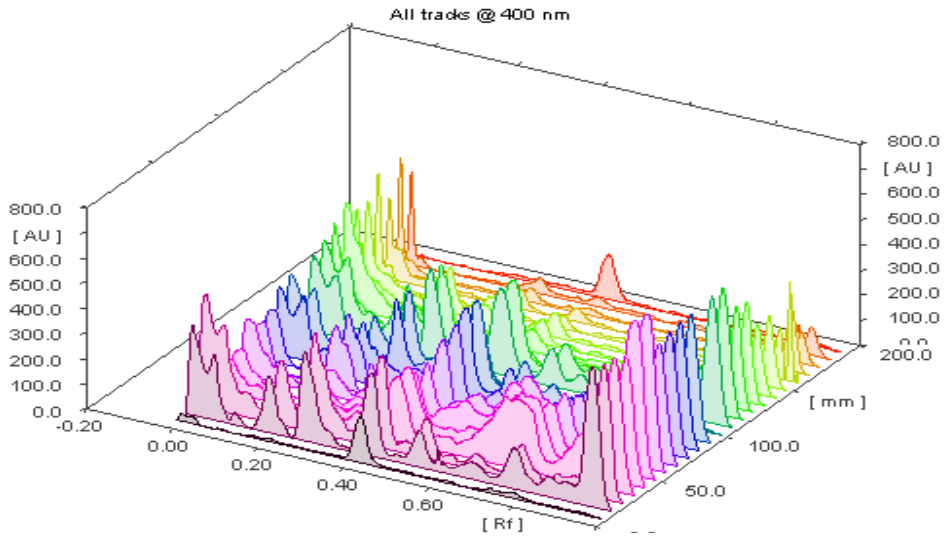

Figure 5. 3D display of all samples at 400 nm.

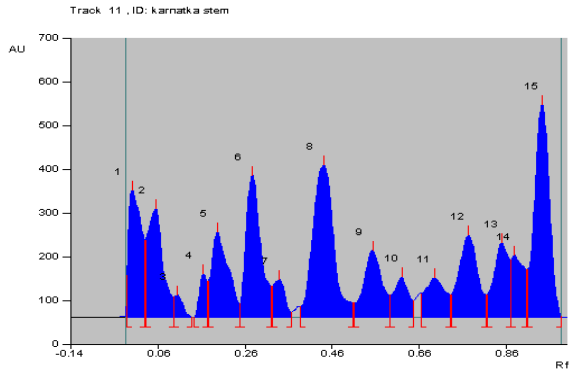

Figure 6. HPTLC fingerprinting of Karnataka stem

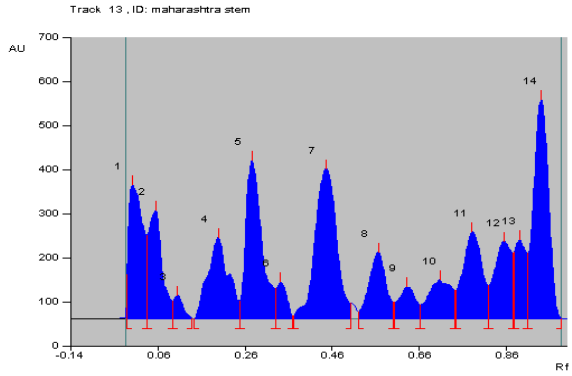

Figure 7. HPTLC fingerprinting of Maharashtra stem

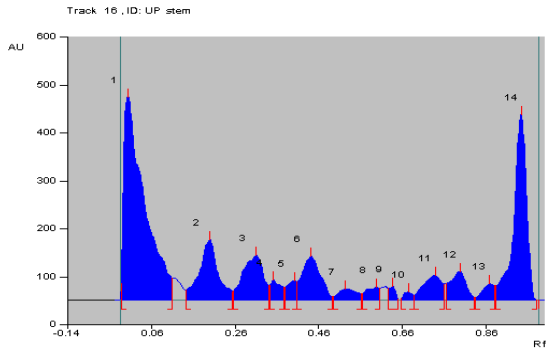

Figure 8. HPTLC fingerprinting of U P stem

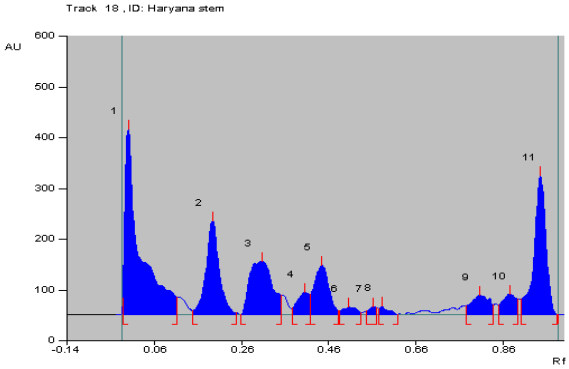

Figure 9. HPTLC fingerprinting of Haryana stem

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