Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Central European Journal of Experimental Biology, 2012, 1 (1):18-25
(http://scholarsresearchlibrary.com/archive.html)

ISSN: 2278–7364

Comparative pharmacognostical evaluation and HPTLC fingerprinting of *Nicotiana tabacum (Linn.)* root collected from different geographical regions of India

Kaushik Sunil[1*], Khan Nazeerullah [2], Ahmad Sayeed[3], Singh Mahaveer[3], Gupta Sumit[4] Semwal Amit[5], Majumdar Manish[1], Acharya Mrityunjoy[1]

[1]Department of Pharmacognosy, GRD (PG) Institute of Management & Technology, Dehradun, INDIA
[2]Department of Pharmacognosy, Dr. KNMIPER, Modi Nagar, Dist. Ghaziabad, Uttar Pradesh, INDIA
[3]Department of Pharmacognosy and Phytochemistry, Hamdard University, New Delhi, INDIA
[4]Department of Pharmacognosy, RPIIT, Karnal, Haryana, INDIA
[5]Faculty of Pharmacy, GISIPS, Dehradun, INDIA

ABSTRACT

Nicotiana tabacum (Linn.) 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 root of Nicotiana tabacum (Linn.). Pharmacognostic evaluation included examination of morphological and microscopical characters; physicochemical properties, phytochemical analysis, and HPTLC fingerprint. The powder microscopy showed the presence of Cork cells, Pitted vessel, Compound starch grains, acicular and prismatic calcium oxalate crystals. The phytochemical screening revealed the presence of 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.

Key Words: *Nicotiana tabacum* (Linn.), pharmacognostic evaluation, phytochemical screening, HPTLC fingerprint.

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* (Linn.) 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 root; physicochemical parameters like ash values, extractive values, moisture content etc. has been evaluated for different samples of root collected from different geographical regions and preliminary phytochemical analysis of different samples of *Nicotiana tabacum* (Linn.) root 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* (Linn.) 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 roots of *Nicotiana tabacum* (Linn.) 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 root 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 root 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 system 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 10µl for root 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 Rf 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 DISSCUSSION

Macroscopic Characters of the root: The root of *Nicotiana tabacum* (Linn.) is light yellow in color, faint in odor, bitter in taste, 10-20cm in length and 2-5 mm in width. It is tapering with numerous rootlets (Figure 1).

Microscopic characters of the root: The transverse section of mature root presents a circular outline with following important tissue from periphery to centre. (Figure 2. a, b, c, d).
Cork: 5-6 layered. Thick walled tangentially elongated cells with brownish pigments.

Cortex: upper 1/3 part composed of 3-4 layered collenchymatous cells, remaining portion composed of parenchymatous cells.
Pericycle: multilayered lignified scherenchymatous cells, forming a continuous circle of arches.
Vascular bundle: open and collateral and consists of:
Phloem: appears as cap over the metaxylem
Xylem: wedge shaped patches separated by multiseriate medullary rays. Large number of lignified pitted xylem vessels. Xylem vessels and xylem parenchyma are prominent.
Medullary rays: appear like spokes of wheel. Thin walled parenchymatous cells packed with starch grains.
Pith: small central parenchymatous portion.

Powder microscopy: Powder microscopy of *Nicotiana tabacum* (Linn.) root revealed following characters: Cork cells, Pitted vessel, Compound starch grains and Acicular and prismatic calcium oxalate crystals (Figure 3. a, b, c, d).

Preliminary Phytochemical Screening: For the phytochemical studies, the preliminary phytochemical screening of petroleum ether, chloroform, ethanol and aqueous extract for *Nicotiana tabacum* (Linn.) root have been done. The result revealed the presence of 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 (Table 2). 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 (Table 3). Significant amount of moisture have also been found in air dried materials of *Nicotiana tabacum* (Linn.) (Table 4). Foreign organic matter was also calculated which is useful tool in detection of contaminant which may be sand, soil, stone, dust or animal excreta (Table 5). 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.

HPTLC fingerprinting of root
HPTLC fingerprinting was carried out for Karnataka and Haryana root samples in petroleum ether extract after spraying with anisaldehyde sulphuric acid reagent using CAMAG HPTLC system, so many phytochemical variations were observed in chromatogram (Figure 4, 5, 6). The petroleum ether extract of Karnataka root sample at 400nm showed the presence of highest no of compounds (Table 6) 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.

Fig. 1: *Nicotiana tabacum* (Linn.) root

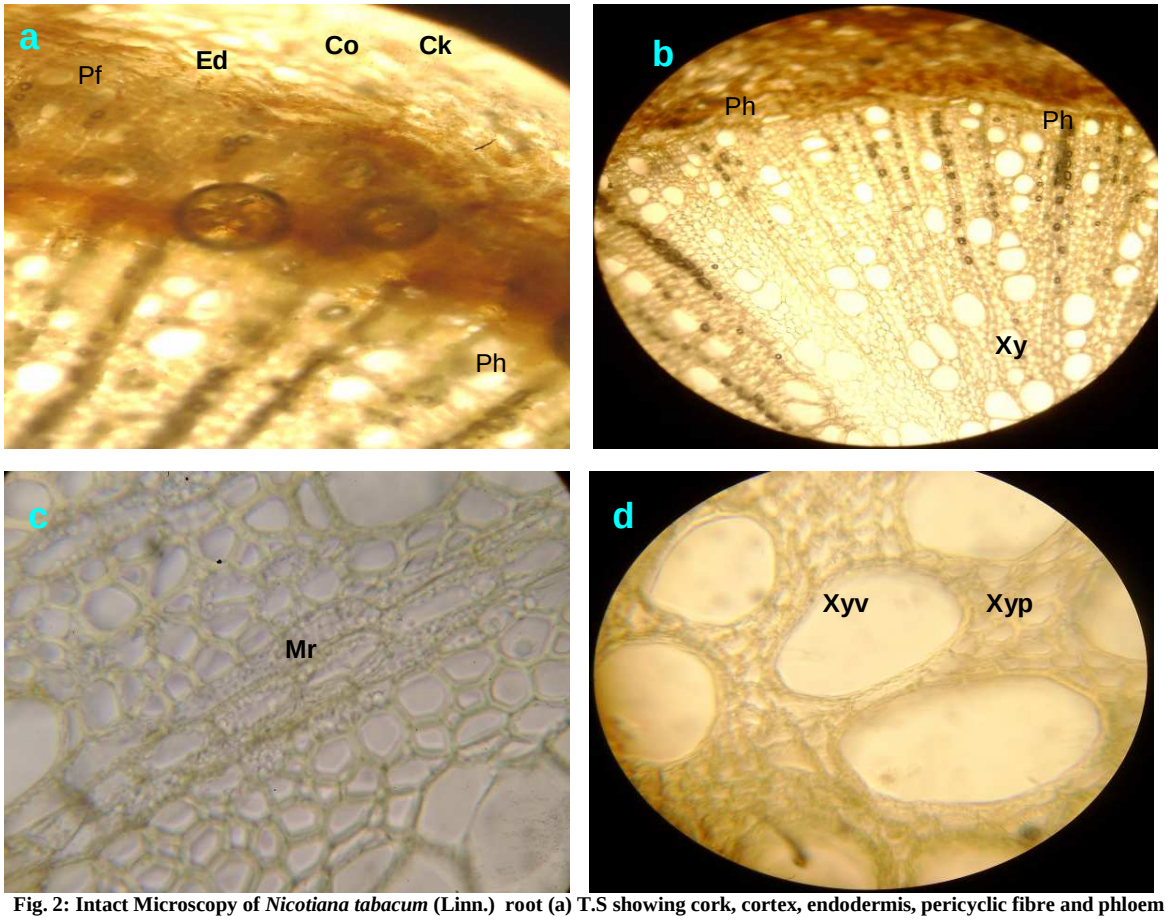


Fig. 2: Intact Microscopy of *Nicotiana tabacum* (Linn.) root (a) T.S showing cork, cortex, endodermis, pericyclic fibre and phloem (b) T.S showing xylem and phloem (Xy, Ph) (c) T.S showing medullary rays (Mr) (d) T.S showing xylem vessel and xylem parenchyma cells (Xyv, Xyp).

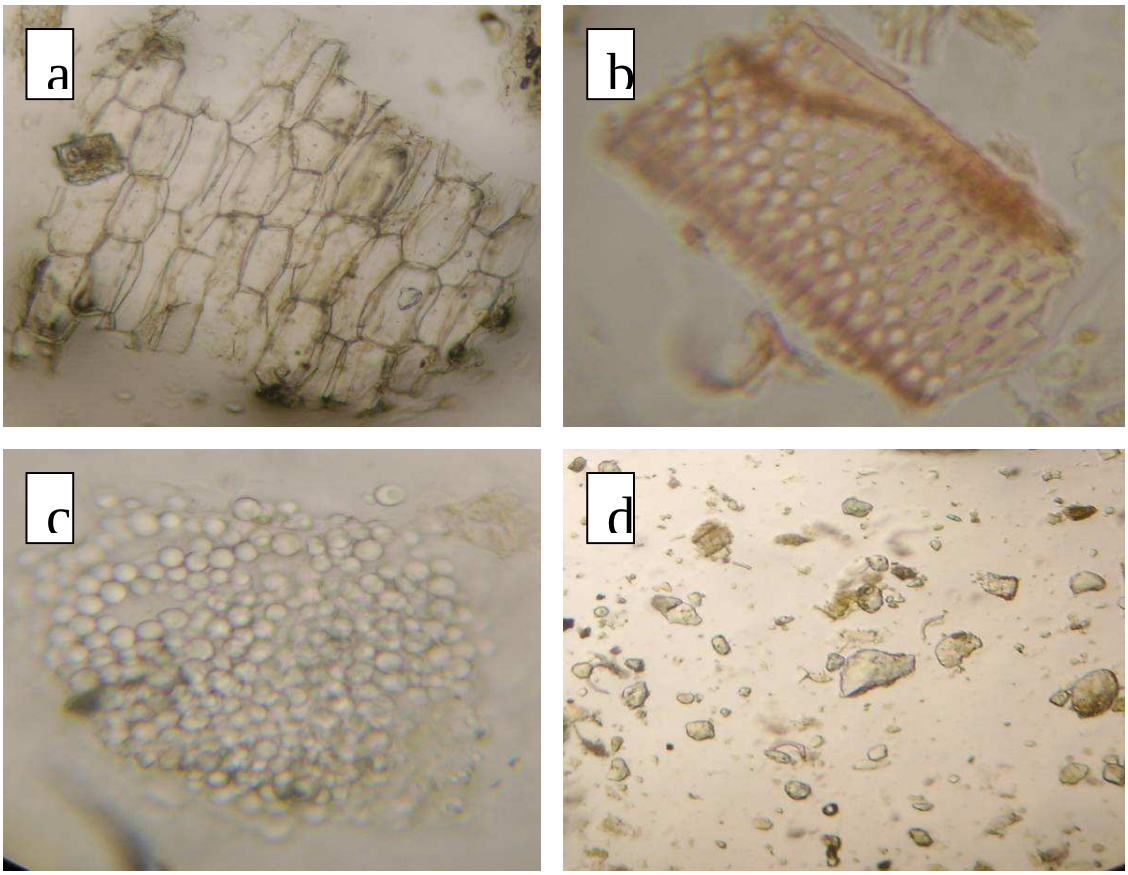
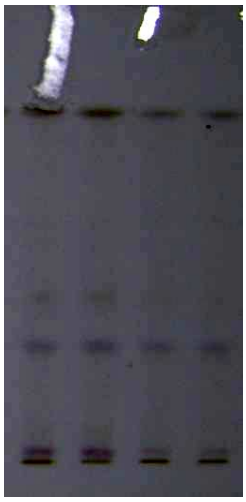


Fig. 3: Powder microscopy of *Nicotiana tabacum* (Linn.) root (a) cork cells (b) Pitted vessel (c) Compound starch grains (d) calcium oxalate crystals.



KR HR
(Root sample)

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

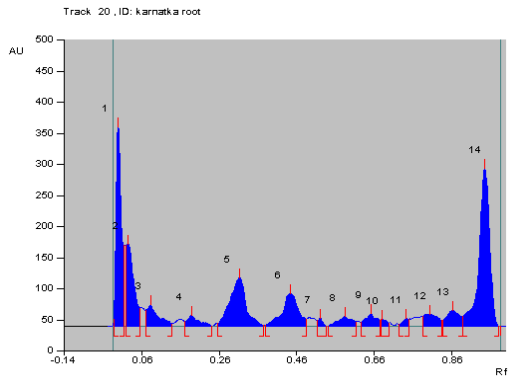


Fig. 5: HPTLC fingerprinting of Karnataka root

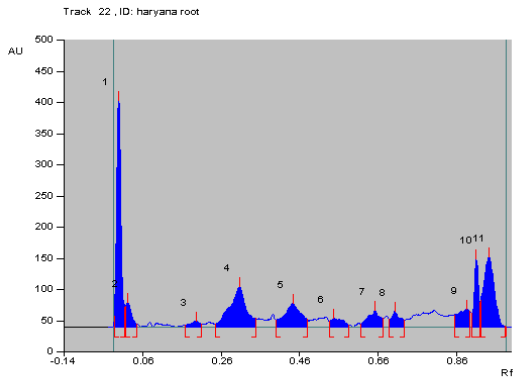


Fig. 6: HPTLC fingerprinting of Haryana root

Table. 1: Phytochemical screening of root extracts of *Nicotiana tabacum* (Linn.)

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: Ash value determination of *Nicotiana tabacum* (Linn.) root of different geographical region

Region of root sample	Ash parameter	Ash values(% w/w)
Haryana	Total ash	12.69±0.095
	Water soluble ash	9.98±0.08
	Acid insoluble ash	1.74±0.05
Karnataka	Total ash	15.03±0.62
	Water soluble ash	12.93±1.19
	Acid insoluble ash	1.71±0.13
Maharashtra	Total ash	12.3±0.12
	Water soluble ash	9.14±0.23
	Acid insoluble ash	1.57±0.09
Uttar Pradesh	Total ash	12.28±0.08
	Water soluble ash	10.68±0.42
	Acid insoluble ash	2.19±0.05

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

Table. 3: Extractive value for successive extraction of solvent in *Nicotiana tabacum* (Linn.) root of different geographical region

Region of root sample	Solvent	Extractive values (% w/w)
Haryana	Pet. Ether	0.51±0.075
	Chloroform	0.26±0.043
	Ethanol	2.52±0.19
	Water	2.15±0.09
Karnataka	Pet. Ether	0.97±0.01
	Chloroform	0.15±0.019
	Ethanol	3.66±0.125
	Water	2.7±0.08
Maharashtra	Pet. Ether	0.45±0.01
	Chloroform	0.12±0.01
	Ethanol	2.27±0.09
	Water	2.46±0.098
Uttar Pradesh	Pet. Ether	0.71±0.08
	Chloroform	0.39±0.05
	Ethanol	5.81±0.105
	Water	4.67±0.12

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

Table. 4: Loss on drying for *Nicotiana tabacum* (Linn.) root samples

Region of root sample	% LOD
Haryana	6.56±0.19
Karnataka	5.53±0.25
Maharashtra	6.37±0.13
Uttar Pradesh	6.44±0.37

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

Table. 5: foreign organic matter in *Nicotiana tabacum* (Linn.) root samples

Region of root sample	% foreign organic matter
Haryana	3.35±0.32
Karnataka	2.18±0.19
Maharashtra	1.67±0.104
Uttar Pradesh	0.87±0.15

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 number	Region	Number of peaks	Corresponding Rf values
1	Karnataka(root)	11	0.19, 0.31, 0.44, 0.52, 0.58, 0.65, 0.68, 0.74, 0.8, 0.86, 0.94
2	Haryana(root)	9	0.19, 0.31, 0.44, 0.54, 0.65, 0.7, 0.88, 0.91, 0.94

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.

REFERENCES

[1] R Chaudhay; Herbal Medicine for Human Health, (Regional Publication SEARO, No. 20, W.T.O New Delhi **1992**; pp. 80.
[2] W.H.O, Quality Control Method for Medicinal Plant Materials, Geneva **1998**
[3] PD Sethi, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations, (CBS Publishers and Distributers, New Delhi **1996**; pp. 10-60.
[4] PS Vaidyaratnam, Indian Medicinal Plants, **1990**
[5] KR Kirtikar and BD Basu, Indian Medicinal Plants, International book Publisher, Dehradun, **1991**; pp. 1798-1800.
[6] KM Nadkarni, Indian Materia Medica, Popular Prakashan, **1996**; pp. 853-854.
[7] The wealth of India. Raw materials, Council of Scientific and Industrial Research, New Delhi **2001**; pp. 25-41.

[8] KR Brain, TD Turner, The practical evaluation of phytopharmaceuticals, Wright- Scientechnica, Bristol, **1975**; pp. 4-9.
[9] PK Mukharjee, Quality Control of Herbal Drugs; An Approach to Evaluation of Botanicals. Business Horizones, New Delhi, **2002**; pp. 390-403.
[10] KR Khandelwal, Practical Pharmacognosy. Nirali Prakashan, **2002**; pp. 9-22.
[11] CK Kokate, AP Purohit and SB Gokhale, Pharmacognosy, Nirali Prakashan, Pune, **2003.**