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Preliminary phytochemical screening and HPTLC fingerprinting of *Nicotiana tabacum* leaf.

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ABSTRACT

The leaf of *nicotiana tabacum* was collected, gabbled, pulverized air dried and subjected to gradient extraction with soxhlet apparatus and the different extracts were screened phytochemically for its chemical components. This revealed the presence of alkaloids, reducing compounds (carbohydrates),, flavonoids, saponins, terpenes and steroids in moderate concentration. hptlc fingerprintint parameters had been developed for alcoholic leaf extract of *nicotiana tabacum*. At wavelength (550 nm), resolution was better for this extracts and hence, this wavelength can be taken for obtaining optimum hptlc fingerprinting for this medicinal plant.

Key words: *Nicotiana tabacum*, HPTLC fingerprinting, leaf extract, phytochemical screening

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].

Nicotiana tabacum belonging to family solanaceae is a stout, viscid annual herb upto1-3 m in height and is cultivated through out India. It is known as Tamaku in Hindi and Hogesoppu in Kannada. Traditionally it has been used to treat skin diseases, local infections, bronchitis, asthma and inflammation. in this present study the Preliminary phytochemical screening of *nicotiana tabacum* has been done to identify the chemical constituents and HPTLC fingerprinting of has been performed which may be used as markers for quality evaluation, and standardization of the drug.[4-7]

MATERIALS AND METHODS

Plant material

The Fresh leaves of *Nicotiana tabacum* plants were collected Shimoga distt. Karnataka the plant was identified by local farmers of shimoga and authenticated by Dr.R.Manjunath, Professor, Department of Botany, D.V.S College of Art & Science, Shimoga.

Preparation and Extraction of Plant material

The Fresh leaves of *Nicotiana tabacum* were gabled for removal of adulterants and pulverized. It was air dried at room temperature. For phytochemical screening, 10 g of material was successively submitted to reflux extraction for one hour with 100 mL of petroleum ether, chloroform, ethanol and distilled water. Each extract was analyzed by specific reactions, as described by Schabra et al [8].

HPTLC profile (High Performance Thin Layer Chromatography)

Sample preparation- The dried and powdered leaf sample was sonicated with alcohol (25ml) for thirty minutes. The alcoholic extract obtained was evaporated to dryness in china dish on water bath to get the residue. Each extract residue was re dissolved in 1ml of chromatographic grade alcohol, which was used for sample application on pre-coated silica gel 60F254 aluminium sheets.

Developing solvent system-A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent n Hexane: ethyl acetate (5:1)

Sample application- Application of bands of each extract was carried out (4mm in length and 1ul in concentration for leaf) using spray technique. Sample were applied in duplicate on pre-coated silica gel 60F254 aluminium sheets (3 x 10 cm) 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 550 nm after spraying with anisaldehyde sulphuric acid The R_f values and finger print data were recorded by WIN CATS software.

Phytochemical analysis-Each extract fraction (petroleum ether, chloroform, ethanol and distilled water) was analyzed by specific reactions, as described by Schabra et al[8].The color intensity of extracts and the appearance of solids in them during the identification reactions allow establishing a semi-quantitative presence of the second-

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

Chemical Constituent	Tests	Pet.ether Ext.	Chloroform Ext.	Alcoholic Ext.	Aqueous 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
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)

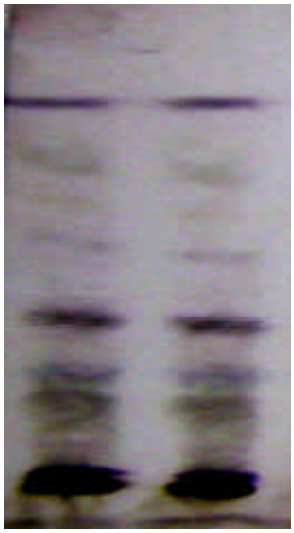

Fig: 1 HPTLC chromatogram of leaf sample of *Nicotiana tabacum* at 550nm in alcoholic extract (anisaldehyde sulphuric acid sprayed data). Sample has been applied in doublet.

ary metabolites[9].

RESULTS AND DISCUSSION

A preliminary phytochemical screening of leaves of *Nicotiana tabacum*

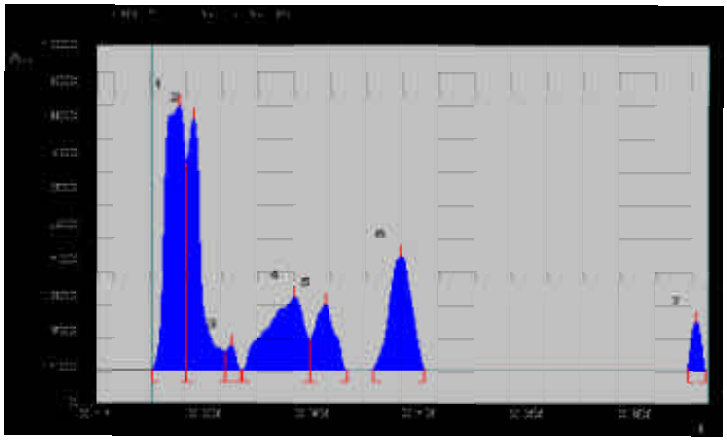

Fig: 2 HPTLC chromatogram of alcoholic extract at 550nm, showing different peaks (bands) of phytoconstituents.

yielded alkaloids, phenols, flavonoids, phytosterols, triterpinoids, tannins and carbohydrates. However, saponins were not detected in leaf part of the plant (Tables1) Triperpenes/steroids were generally identified in the non-polar extracts of leaves. Tannins, phenols and flavonoids were present only in alcoholic and aqueous extracts of leaf. Alkaloids were identified in both polar and non polar extracts of the plant.

In this study the HPTLC fingerprinting of alcoholic extract revealed seven spots at the following Rf values 0.11, 0.23, 0.29, 0.43, 0.98(Peak at Rf value < 0.1 is omitted) 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 corresponding HPTLC chromatograms are presented in Fig. 1 and 2.

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