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Chromatographic finger print analysis of *Naringi crenulata* by HPTLC technique

Subramanian Sampathkumar[1*], Ramakrishnan N[2]

[1]*Department of Botany Government Arts College, Tiruvannamalai, Tamilnadu, India, 606 601*
[2]*Department of Botany, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India, 612 001*

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ABSTRACT

Objective: To establish the fingerprint profile of *Naringi crenulata* (*N. crenulata*) (Roxb.) Nicols. using high performance thin layer chromatography (HPTLC) technique. **Methods:** Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software was used. **Results:** The results of preliminary phytochemical studies confirmed the presence of protein, lipid, carbohydrate, reducing sugar, phenol, tannin, flavonoid, saponin, triterpenoid, alkaloid, anthraquinone and quinone. HPTLC finger printing of ethanolic extract of stem revealed 10 spots with R_f values in the range of 0.08 to 0.65; bark showed 8 peaks with R_f values in the range of 0.07 to 0.63 and the ethanol extract of leaf revealed 8 peaks with R_f values in the range of 0.09 to 0.49, respectively. The purity of sample was confirmed by comparing the absorption spectra at start, middle and end position of the band. **Conclusions:** It can be concluded that HPTLC finger printing of *N. crenulata* may be useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies.

1. Introduction

Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries[1]. 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 technique and applying suitable standards[2]. High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images[3].

Naringi crenulata (*N. crenulata*) (Roxb.) Nicols. (Family: Rutaceae) is commonly known as Mahavilvam in Tamil and it is distributed throughout India, especially in the Southern Western Ghats, South and Central Sahyadris and Indomalysia[4]. All parts of this tree *viz.* root, stem, bark, leaf and fruit have been used in folk medicine[5]. Root is used as remedy for cobrabite[6], bodypain[7], colic[8], vomiting and dysentery[9]. Stem powder prevents acne and has anti-aging property[10]. Bark is used as a remedy for puerperal fever[11], pitta[11] and bark juice is applied externally for getting speedy relief in sprain[13]. Traditionally, leaves are used for curing mental disorders[14], dysentery[11], digestive disorders[15], fever[16] and epilepsy[12]. Fruit decoction is used as antidote to insect poison[8], and intestinal worms[17], and also has anthelmintic property[11].

Preliminary phytochemical screening of *N. crenulata* has been done to identify the chemical constituents and HPTLC finger printing has been performed which may be used as

*Corresponding author: Subramanian Sampathkumar, Department of Botany Government Arts College, Tiruvannamalai, Tamilnadu, India, 606 601.
Tel: 09842326180
E-mail: sampathbio@gmail.com

a marker for quality evaluation, and standardization of the drug.

2. Materials and methods

2.1. Plant material

The plant specimen was collected from Adiannamalai region of Tiruvannamlai, Tamilnadu, India. The taxonomic identification of the plant was confirmed by Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India. (Certificate No. BSI/SRC/5/23/10-11/Tech.1134) and the voucher specimen was deposited in the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Tamilnadu, India.

2.2. Preparation and extraction of plant material

The collected fresh plant parts were air-dried at room temperature. The dried plant material was then homogenized by electric mixer grinder to obtain coarse powder and stored in air-tight bottles for further analysis. The shade dried, powdered samples were extracted with 150 mL of solvent ethanol for 8-12 h by using the Soxhlet apparatus[18].

2.3. Phytochemical screening

Preliminary phytochemical screening was done following the method of Kokate[19] and Khandelwal[20].

2.4. HPTLC profile

HPTLC studies were carried out following the method of Harborne[21] and Wagner *et al*[22].

2.4.1. Sample preparation

The ethanolic bark, stem and leaf extracts were dissolved in HPTLC grade ethanol which were used for sample application on precoated silica gel GF254 aluminium sheets.

2.4.2. Developing solvent system

A number of solvent systems were tried, for extract, but the satisfactory resolution was obtained in the solvent toluene-ethyl acetate-formic acid (7:3:0.1).

2.4.3. Sample application

The samples (5 μL) were spotted in the form of bands of width 6 mm with a 100 μL sample using a Hamilton syringe on silica gel which was precoated on aluminum plate GF-254 plates (20 cm × 10 cm) with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software.

2.4.4. Development of chromatogram

The mobile phase consisted of toluene-ethyl acetate-formic acid (7:3:0.1) and 15 mL of mobile phase was used per chromatography run. The linear ascending development was carried out in a (20 cm × 10 cm) twin through glass chamber saturated with the mobile phase.

2.4.5. Detection of spots

The developed plate was dried by hot air to evaporate solvents from the plate. The developed plate was sprayed with anisaldehyde sulphuric acid reagent as spray reagent and dried at 100 ℃ in hot air oven for 3 min. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under UV light at 254 and 334 nm, respectively. The R_f values and finger print data were recorded by WIN CATS software.

3. Results

A preliminary phytochemical screening of leaves, stem and bark of *N. crenulata* yielded protein, lipid, carbohydrate, reducing sugar, phenol, tannin, flavonoid, saponin, triterpenoid, alkaloid and quinone. However, alkaloid showed negative results in bark and leaf. Triterpenoid also showed negative results in stem and leaf. Anthraquinone was not detected in leaf, stem and bark of the plant (Table 1).

Table 1
Phytochemical screening of ethanolic extract of stem, bark and leaf of *N. crenulata*.

Chemical constituents	Stem	Bark	Leaf
Protein	++	++	++
Lipid	+	+	++
Carbohydrate	+++	+++	−
Reducing sugar	+++	+++	−
Phenol	+++	+++	+++
Tannin	+	+	+
Flavonoid	+	+	+
Saponin	++	++	++
Triterpenoid	−	++	−
Alkaloid	++	−	−
Anthraquinone	−	−	−
Quinone	−	+	+

+++: Presence; −: Absence.

In this study HPTLC fingerprinting of ethanolic extract of *N. crenulata* of stem, bark and leaves revealed several peaks and was recorded in Table 2. HPTLC profile under UV 366 and 254 nm was recorded in the Figure 1. The corresponding HPTLC chromatograms were presented in Figure 2-4. The stem extract revealed 10 spots with R_f values in the range of 0.08 to 0.65 (Table 2 and Figure 3). Bark extract showed 8 peaks with R_f values in the range of 0.07 to 0.63 (Table 2 and Figure 4) and the ethanolic extract of leaf revealed 8 peaks with R_f values in the range of 0.09 to 0.49 (Table 2 and Figure 5) and purity of the sample was confirmed by comparing the

absorption spectra at start, middle and end position of the band.

Table 2
HPTLC profile of the ethanolic extract of *N. crenulata* (stem, bark and leaf).

Parts of *N. crenulata*	Peak	R_f	Height	Area
Stem	1	0.08	0.90	1 672.50
	2	0.12	5.80	244.40
	3	0.19	20.30	400.10
	4	0.22	21.70	707.20
	5	0.28	4.50	1 196.70
	6	0.36	0.00	242.00
	7	0.42	11.40	315.50
	8	0.48	42.80	1 178.60
	9	0.58	9.90	2 735.80
	10	0.65	2.70	556.30
Bark	1	0.07	0.80	679.20
	2	0.09	8.50	99.20
	3	0.13	0.10	320.50
	4	0.23	33.50	7 807.00
	5	0.28	0.10	1 909.50
	6	0.44	18.90	5 030.50
	7	0.52	18.80	2 480.10
	8	0.63	3.10	2 631.10
Leaf	1	0.09	12.20	5 514.20
	2	0.12	8.90	653.30
	3	0.15	0.00	310.20
	4	0.23	79.90	2 497.80
	5	0.28	0.50	3 816.80
	6	0.37	0.20	2 167.80
	7	0.44	19.90	1 149.60
	8	0.49	41.80	1 207.00

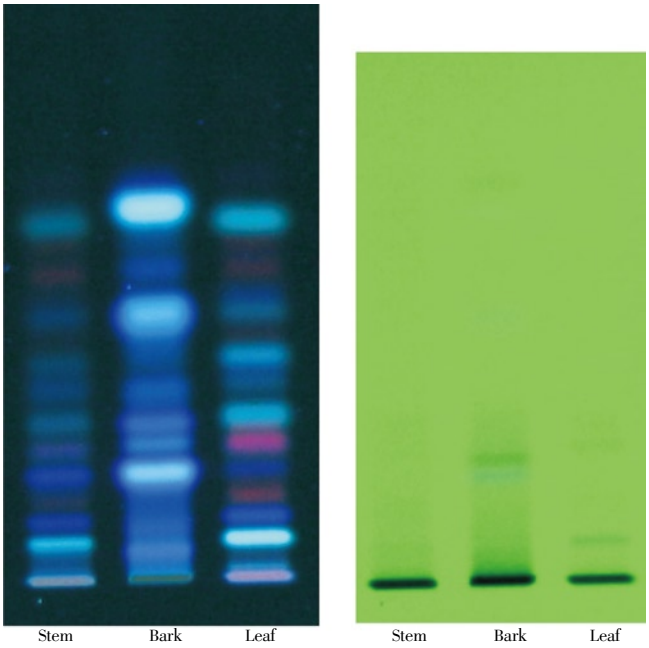

Figure 1. HPTLC profile of the ethanolic extract of *N. crenulata* under UV 366 and 254 nm.

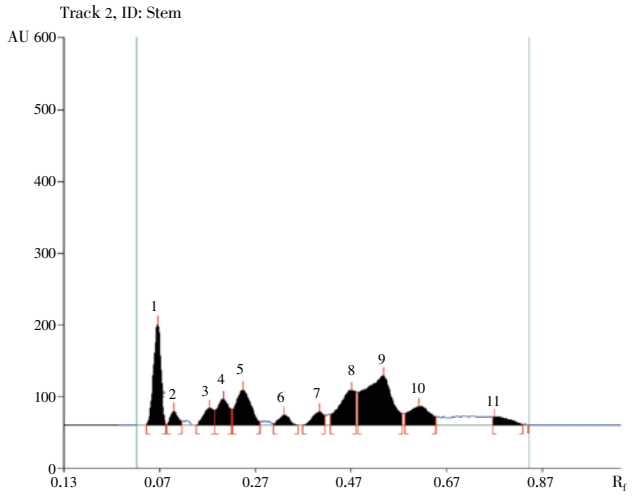

Figure 2. HPTLC chromatogram of *N. crenulata* stem extract showing different peaks of phytoconstituents.

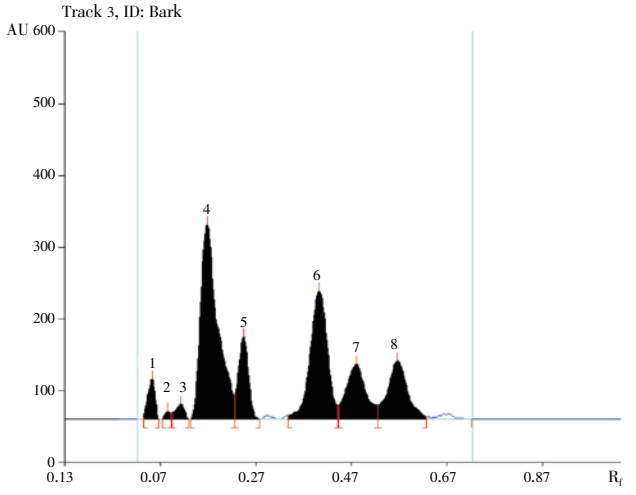

Figure 3. HPTLC chromatogram of *N. crenulata* bark extract showing different peaks of phytoconstituents.

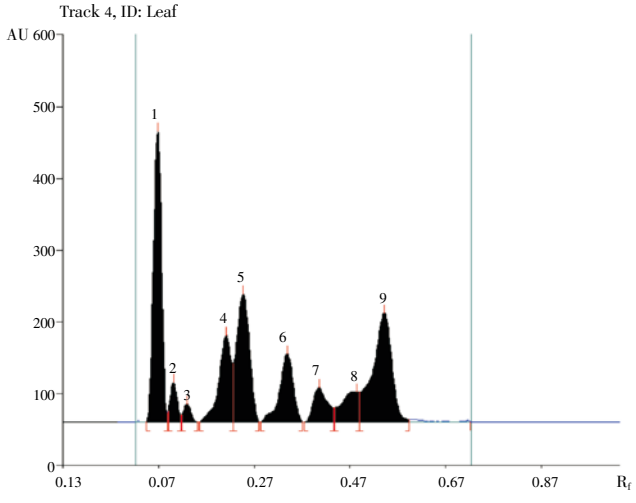

Figure 4. HPTLC chromatogram of *N. crenulata* leaf extract showing different peaks of phytoconstituents.

4. Discussion

Authentication of medicinal plants as genetic and chemical level is a critical step in the use of these botanical materials for both research purposes and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy, during this molecular era, the morphological characters also play a vital role in plant systematic study and are used as a tool for the classification of a taxon. In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organisms. HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies[3].

HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and 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 perform 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.

Conflict of interest statement

We declare that we have no conflict of interest.

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