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Research Article

HPTLC Fingerprint Analysis of *Naregamia alata* W&A- A Medicinal Plant

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ABSTRACT

Aim of this study was to develop a HPTLC fingerprint profiles for various secondary metabolites of methanol extract of the root of the traditional medicinal plant, *Naregamia alata*. CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 and WIN CATS-4 software were used. HPTLC finger printing of methanol extract of root carried out in three different mobile phases, which showed different Rf values. HPTLC finger printing of methanol extract of root in Mobile phase1 revealed 10 peaks with Rf values in the range of 0.13 to 0.92, Mobile phase 2 showed 7 peaks with Rf values in the range of 0.27 to 0.93 and Mobile phase 3 revealed 4 peaks with Rf values in the range of 0.25 to 0.78. It can be concluded that different Rf values of various phytochemicals provide valuable idea regarding their polarity and selection of solvents for separation of phytochemicals. This study will help in future for identifying this plant for further research.

Keywords: *Naregamia alata*, HPTLC analysis, Meliaceae.

INTRODUCTION

India has one of the oldest, richest and different cultural traditions associated with the use of medicinal plants. Medicinal plants are traditionally used for thousands of years and they are present in a group of herbal preparations of the Indian traditional health care system, and proposed for their interesting activities. WHO has emphasized the need to ensure the quality of medicinal plant products using modern techniques and suitable standards[1]. HPTLC offers better resolution and estimation of active constituents with reasonable accuracy in a shorter time.

Naregamia alata W&A commonly known as 'Nilanarakam' belonging to the family Meliaceae. It is a small branching undershrub and used traditionally for its curative property in treating anaemia, enlarged spleen, ulcers, rheumatism, malaria and acute dysentery. Root contains an alkaloid 'Naregamin', and other chemical constituents are Hannesane, β sitosterol, palmitic and steric acid[2]. The plant is reported to contain phenol, flavonoids, alkaloids, terpenoids and tannins. In this present study the Preliminary phytochemical screening of *Naregamia alata* has been done to identify the chemical constituents and HPTLC fingerprinting provide quantitative information about the main active constituents or marker compounds present in the crude herbal products and it can be used as a diagnostic tool for the correct identification of the plant.

MATERIALS AND METHODS

Plant material

The plant specimen for the proposed study was collected from Elavoor, Ernakulam district, Kerala. The plant was authenticated by Dr. N. Mohanan Scientist, JNTBGRI Palode. The voucher specimen of the plant [Vocher No. 66911] was prepared and deposited at TBGT for further references.

HPTLC profile (High Performance Thin Layer Chromatography

HPTLC studies were carried out following the method[3,4]

Sample preparation

The 50gms of coarsely powered plant root of *Naregamia alata* was defatted with petroleum ether and extracted with methanol using Soxhlet apparatus Methanol extract obtained was evaporated under reduced pressure using rotary evaporator. 1mg Extract residue was redissolved in 1ml of chromatographic grade methanol, which was used for sample application on pre-coated silica gel 60F 254 aluminium sheets.

Developing solvent system

Habit of *Naregamia alata* W & A.

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Table 1: Rf values for methanol root extract-Solvent system Toluene:n Butanol (4.5:0.5).

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.08 Rf	0.7 AU	0.11 Rf	18.8 AU	5.27 %	0.13 Rf	11.3 AU	335.0 AU	3.66 %	unknown *
2	0.13 Rf	11.4 AU	0.17 Rf	34.5 AU	9.64 %	0.19 Rf	22.5 AU	767.3 AU	8.38 %	unknown *
3	0.19 Rf	22.8 AU	0.19 Rf	24.7 AU	6.90 %	0.23 Rf	10.3 AU	430.2 AU	4.70 %	unknown *
4	0.24 Rf	10.6 AU	0.25 Rf	15.9 AU	4.45 %	0.29 Rf	3.6 AU	285.6 AU	3.12 %	unknown *
5	0.35 Rf	1.7 AU	0.39 Rf	10.8 AU	3.03 %	0.40 Rf	6.4 AU	195.7 AU	2.14 %	unknown *
6	0.43 Rf	5.6 AU	0.45 Rf	14.8 AU	4.13 %	0.48 Rf	0.2 AU	263.1 AU	2.87 %	unknown *
7	0.64 Rf	4.3 AU	0.69 Rf	24.9 AU	6.98 %	0.70 Rf	21.1 AU	490.4 AU	5.36 %	unknown *
8	0.71 Rf	22.9 AU	0.74 Rf	47.9 AU	13.40 %	0.76 Rf	29.5 AU	1087.5 AU	11.88 %	unknown *
9	0.77 Rf	30.0 AU	0.80 Rf	69.6 AU	19.48 %	0.81 Rf	36.5 AU	1266.8 AU	13.84 %	unknown *
10	0.81 Rf	66.5 AU	0.83 Rf	95.5 AU	26.72 %	0.92 Rf	11.1 AU	4035.1 AU	44.07 %	unknown *

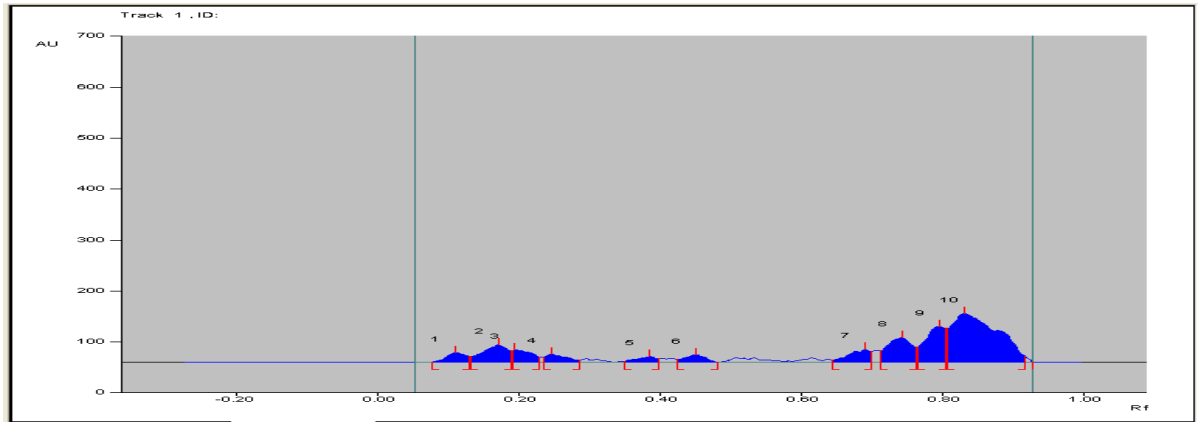

Figure 1: Chromatogram of methanol root extract: Toluene:n Butanol (4.5:0.5).

Table 2: Rf values for methanol root extract-Solvent system Toluene:n Butanol:Formic acid (4.5:0.4:0.1).

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.21 Rf	0.4 AU	0.25 Rf	17.6 AU	7.69 %	0.27 Rf	5.3 AU	327.7 AU	4.05 %	unknown *
2	0.28 Rf	5.8 AU	0.30 Rf	15.7 AU	6.88 %	0.35 Rf	2.4 AU	342.1 AU	4.23 %	unknown *
3	0.48 Rf	2.6 AU	0.51 Rf	12.3 AU	5.39 %	0.54 Rf	4.4 AU	286.1 AU	3.54 %	unknown *
4	0.58 Rf	8.4 AU	0.62 Rf	14.0 AU	6.13 %	0.64 Rf	10.7 AU	410.2 AU	5.07 %	unknown *
5	0.65 Rf	12.5 AU	0.69 Rf	31.5 AU	13.77 %	0.70 Rf	28.2 AU	669.3 AU	8.28 %	unknown *
6	0.71 Rf	28.6 AU	0.74 Rf	44.6 AU	19.47 %	0.75 Rf	39.2 AU	868.4 AU	10.74 %	unknown *
7	0.75 Rf	39.3 AU	0.81 Rf	93.1 AU	40.67 %	0.93 Rf	0.2 AU	5183.0 AU	64.09 %	unknown *

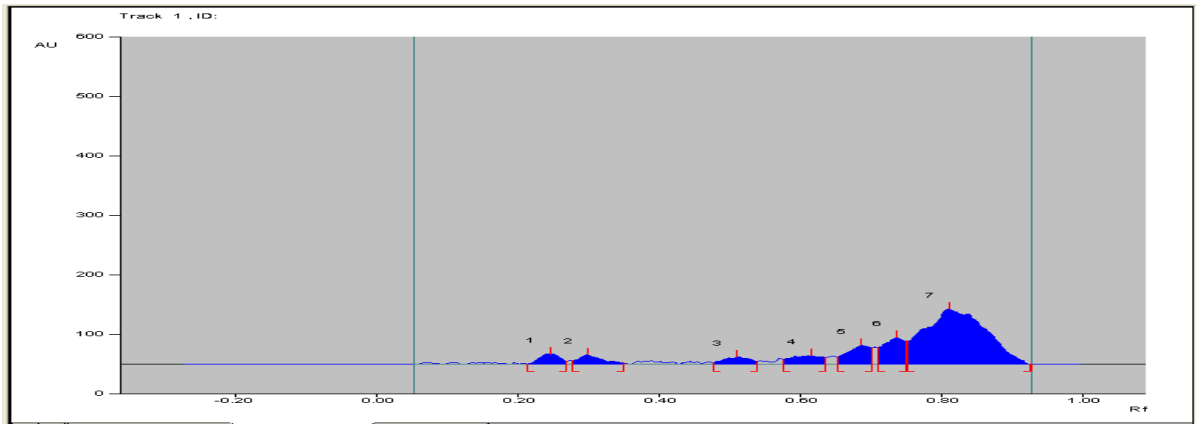

Figure 2: Chromatogram of methanol root extract Toluene:n Butanol:Formic acid (4.5:0.4:0.1).

A number of solvent systems were tried for extract, but the satisfactory resolution was obtained in the 3 different solvent systems. These were Toluene:n Butanol (4.5:0.5), Toluene: Ethylacetate: Formicacid: Methanol (3:3:2:1) and Toluene: n Butanol: Formic acid (4.5:0.4:0.1).

Sample application

Application of bands of root extract was carried out (4mm in length and 1µl in concentration) using spray technique. Sample was applied in duplicate on precoated silica gel 60F254 aluminiun sheets (5×10cm) 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 sample, the chromatogram was developed in Twin trough glass chamber 10×10 cm saturated with different solvents Toluene::n Butanol (4.5:0.5), Toluene: Ethylacetate: Formicacid: Methanol (3:3:2:1) and Toluene: n Butanol: Formic acid (4.5:0.4:0.1) for 15min.

Detection of spots

The air dried plates were viewed in ultraviolet radiation to mid day light. The chromatograms were scanned by densitometer at 254nm. The Rf values and fingerprint data were recorded by WIN CATS software.

RESULTS AND DISCUSSION

Naregamia alata of family Meliaceae is found through out southern region of India. The various parts of the plant have traditional medicinal properties. *Naregamia alata* root is used to cure some of the diseases like rheumatism and acute dysentery. Literature survey said that review was made in this plant and also few pharmacological works like microbiological, hepatoprotective, antioxidant, antiulcer activity. There is no research work on the standardisation and isolation of the active constituent in this plant. The phytochemical test on methanolic root extract showed the presence of alkaloid, glycosides, phenol, flavonoids, terpenoids and tannins, were previously reported. So, the present investigation is an attempt made to study its HPTLC studies.

HPTLC analysis of root extract of N. alata

Qualitative phytochemical analysis provides the information regarding the presence of primary and secondary metabolites of clinical significance. HPTLC techniques were performed on 3 different mobile phases.

The mobile phase 1 selected for the HPTLC studies of methanolic root extract was Toluene:n Butanol in the ratio of (4.5:0.5). There are 10 polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.13 to 0.92 in which highest concentration of the phytoconstituents was found to be 26.72% respectively and was recorded in Table 1. The corresponding HPTLC chromatogram was presented in Figure1.

The mobile phase 2 selected for the HPTLC studies of methanolic root extract was Toluene:n Butanol:Formic acid (4.5:0.4:0.1). There are 7 polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.27 to 0.93 in which highest concentration of the phytoconstituents was found to be 40.67% respectively and was recorded in Table 2. The corresponding HPTLC chromatogram was presented in Figure2.

The mobile phase 3 selected for the HPTLC studies of methanolic root extract was Toluene: Ethyl acetate: Formic acid: Methanol (3:3:2:1). There are 4 polyvalent phytoconstituents and corresponding ascending order of R_f values start from 0.25 to 0.78 in which highest concentration of the phytoconstituents was found to be

Table 3: Rf values for methanol root extract-Solvent system Toluene: Ethyl acetate: Formic acid: Methanol (3:3:2:1).

Track	Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	1	0.12 Rf	3.3 AU	0.19 Rf	153.9 AU	62.55 %	0.25 Rf	33.4 AU	8771.5 AU	70.89 %	unknown *
1	2	0.45 Rf	24.7 AU	0.49 Rf	42.5 AU	17.26 %	0.52 Rf	31.9 AU	1779.0 AU	14.38 %	unknown *
1	3	0.60 Rf	28.4 AU	0.62 Rf	33.9 AU	13.79 %	0.67 Rf	16.6 AU	1313.0 AU	10.61 %	unknown *
1	4	0.71 Rf	8.2 AU	0.74 Rf	15.7 AU	6.40 %	0.78 Rf	4.9 AU	510.7 AU	4.13 %	unknown *

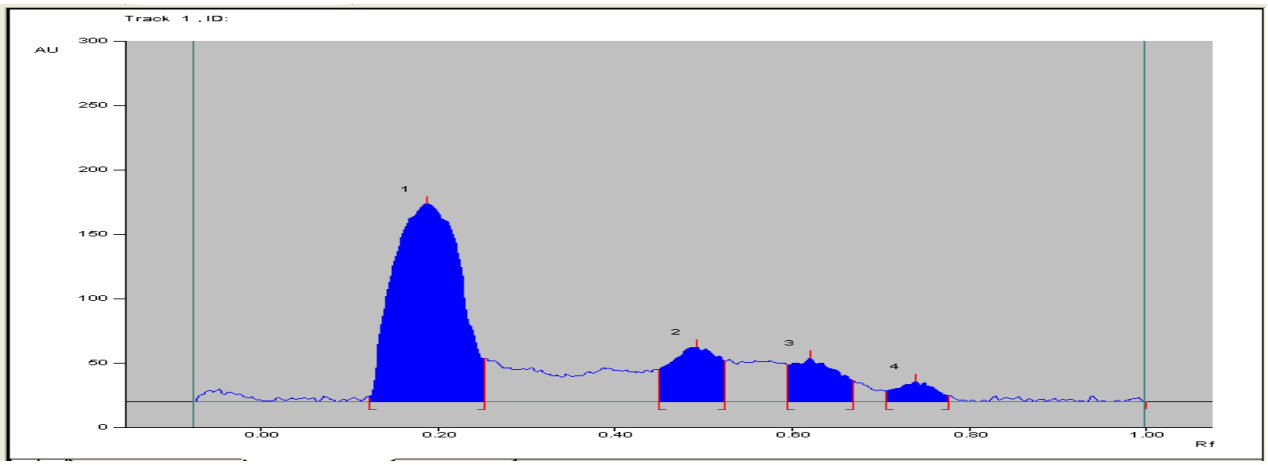

Figure 3: Chromatogram of methanol extract Toluene: Ethyl acetate: Formic Acid: Methanol (3:3:2:1).fig

62.55% respectively and was recorded in Table 3. The corresponding HPTLC chromatogram was presented in Figure3.

The present studies on preliminary phytochemical screening and HPTLC provide useful information which may help in authenticating the plant along with nature of phytoconstituents present in it. HPTLC studies show clear separation of components present in the methanolic root extract of *N. alata*. HPTLC fingerprint enables a particular plant to be identified and distinguished from closely related species[5].

The root extract was screened through HPTLC. The methanolic extract shows 65% constituents. Blue fluorescence was observed on methanolic extract. So, this is a correct extract for the isolation of the active constituents. This extract selected for the isolation studies to develop a new drug molecule in future. It is more useful to cure many diseases with less side effects.

CONCLUSION

Qualitative tests performed on the root extracts of *N. alata* indicate the presence of glycosides, flavonoids, alkaloids and phenol in methanolic root extract. To understand the actual chemical constituents responsible for the pharmacological activities of the plant detailed chromatographic studies are needed to be carried. Present study showed varied composition of methanolic root extract of *N.alata* using the HPTLC technique. In this study multiple peaks correspond to various compounds. Different peaks observed in this technique indicate the diverse composition of the extracts. The data and profile can be used as a valuable analytical tool in the quality control and standardization. Further characterization are necessary to find out the exact chemical constituents and their relation to the pharmacological activity.

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