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Research Article

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Assessment of functional group through Fourier Transformation Infrared Spectroscopy and HPTLC analysis of single drug Naruvili Chooranam (*Cordia dichotoma* Forst.G)

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Abstract

Aim: The aim of the study is to assess the functional group of Naruvili chooranam through FTIR and HPTLC analysis. **Methodology:** Naruvili chooranam was prepared as mentioned in Siddha literature and stored for experimental purpose. FTIR and HPTLC analysis were conducted at Siddha Regional Research Institute, Trivandrum. **Results:** FTIR Analysis of Naruvili chooranam exhibits the peak value at 471, 540, 646, 671,810,841,1003,1122,1664 having C-I stretching, C-Br stretch, C-H bend, C-H out of plane, Si-OR, S=O stretching, C=C stretching. This peak value indicates the presence of halocompounds, alkyl halides, alkynes,

aromatics, sulfoxide and alkenes. HPTLC analysis reveals the presence of versatile phytochemicals. Rf value of the peak ranges from 0.02 to 0.86.**Conclusion:** The results indicates the presence of essential functional group and versatile phytochemicals. This mate it suitable for evaluating the quality characteristics of Naruvili chooranam as a bench mark for creating the standardized pharmaceutical product.

Keywords: Naruvili chooranam, FTIR, HPTLC, Iraippu Noi

1. Introduction

Siddha medicine is an ancient healing system that originated in south india and is regarded as one of the traditional forms of medicine in India. It combines traditional medicinal practices with spiritual disciplines, alchemy and mysticism. Single drug therapy (Eka mooligai Prayogam), is gaining widespread popularity among both the scientific community and public. This is mainly due to its effectiveness, affordability and minimal side effects. Likewise Naruvili chooranam (*Cordia dichotoma* Forst.G) is a single drug preparation indicated for Iraippu Noi (Bronchial asthma). Fourier transformation Infra red spectroscopy is speedy and harmless analytical technique that when combined with chemometrics become the valuable asset for pharmaceutical sector. It detect the presence of different functional groups which showed major compounds present in the leaf extracts. High Performance Thin Layered Chromatography reveals the presence of phytochemicals in the sample and establishes its credibility through a scientific approach.

Kingdom	:	Plantae
Division	:	Magnoliophyta
Subclass	:	Astaridae
Order	:	Lamiales
Family	:	Boranginaceae
Genus	:	Cordia
Species	:	*C. dichotoma* Forst.f
Fragrant	:	Manjack

2. Materials and Methods

2.1. Collection of Raw drug:

Naruvili leaves were collected around the areas of Nagercoil and it was authenticated by the faculties at Department of Gunapadam, GSMC, Palayamkottai.

2.2. Sample Preparation:

It was dried in the shade, ground into coarse powder, filtered to obtain chooranam and then stored in an airtight container labelled as Naruvili Chooranam for experimental use.

2.3. Place of study:

FTIR and HPTLC were conducted at Siddha Regional Research Institute (SRRI), Trivandrum, Kerala.

2.4. FTIR analysis-methodology

The drug was subjected to FT-IR analysis using KBr pressed disk technique on Analytical Technologies FT-IR spectrophotometer (Model: INFRA 3000-50) and the characteristic peaks were detected and recorded.

Results

Figure: 1 FTIR Spectral of Sample Naruvilli chooranam

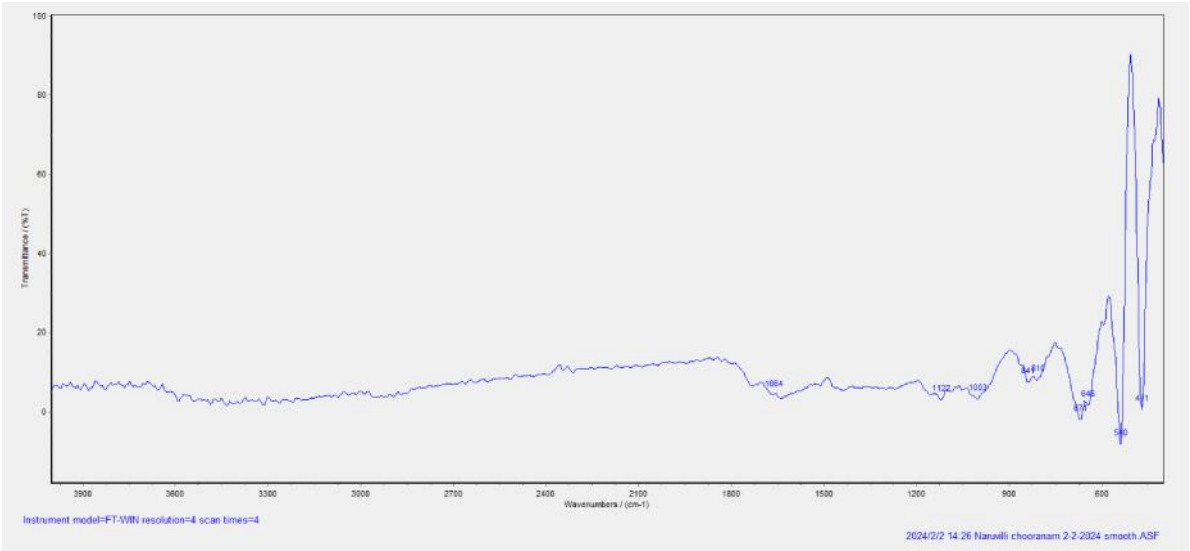


Table: 1. FTIR Spectral of Naruvilli chooranam

S. No	Peak	Characteristic Absorptions (cm^{-1})	Possible Functional Group	Class
1	471	300 – 600	C-I stretching	halo compound
2	540	300 – 600	C-Br Stretch	alkyl halides
3	646	600 – 900	C–H bend	Alkynes
4	671	600 – 900	C–H bend	Alkynes
5	810	600 – 900	C – H out of plane	Aromatics
6	841	600 – 900	C – H out of plane	Aromatics
7	1003	900 – 1200	Si-OR	Misc
8	1122	900 – 1200	S=O stretching	Sulfoxide
9	1664	1500 – 1800	C=C stretching	Alkene

It shows that Naruvili chooranam exhibits the peak value at 471, 540, 646, 671,810,841,1003,1122,1664 having C-I stretching, C-Br stretch, C-H bend, C-H out of plane, Si-OR, S=O stretching, C=C stretching. This peak value indicates the presence of halocompounds, alkyl halides, alkynes, aromatics, sulfoxide and alkenes.

Halo compounds

The antibacterial activities of the Halo complexes were determined against the two Gram positive bacteria: *Staphylococcus aureus* (PTCC1189), Micrococcus and also against the two Gram negative bacteria: *Escherichia coli* (PTCC1329), *Pseudomonas aerusinosa*

Alkyl halides have limited biological effects but are known for their antibacterial and antifungal properties. They are also used as an general anaesthetic in surgical procedures and are important starting material for organic compounds.

Alkynes have antifungal properties and are essential in the pharmaceutical industry, such as being used as a topical analgesic for severe arthritic pain.

Aromatic compounds exhibits antimicrobial, antidiabetic and antioxidant properties.

Sulfoxide can aid in decreasing inflammation and pain and may helps to reduce leakage during chemotherapy. Additionally they possess anti microbial, anti inflammatory, anti oxidant and diuretic properties.

2.5. HPTLC Analysis:

Developing solvent system

A number of solvent systems were tried and a system which gave the maximum resolution was selected as the solvent system for the extract. The optimum separations of constituents were achieved using the solvent system.

Sample application

The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F_{254} pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4).

Development of chromatogram

After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected.

Documentation

The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm.

Densitometry

The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner.

Post chromatographic derivatisation

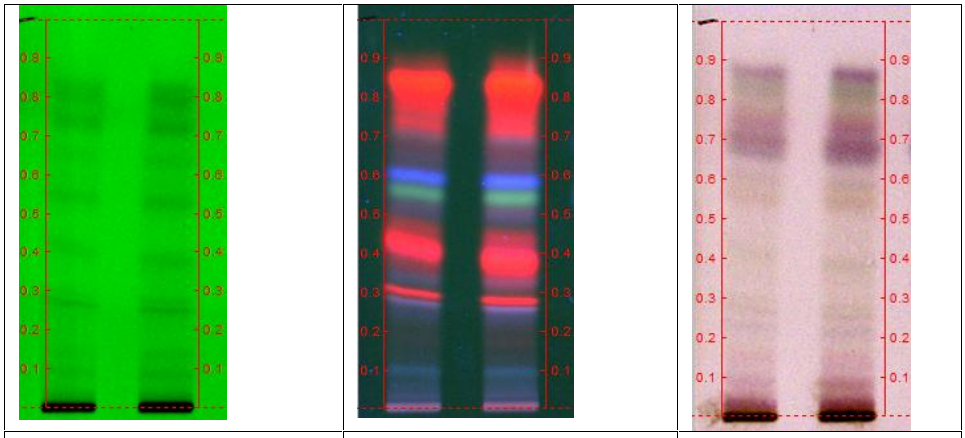
The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0 C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

HPTLC profiling

Solvent system: Toluene: Ethyl acetate: Formic acid (5:7:0.1)

Track 1- 5µl, Track 2- 7µl

Figure 2: HPTLC profile of Naruvilli chooranam



Under UV short	Under UV long	Under white light

Figure 3: HPTLC chromatogram of Naruvilli chooranam at 254nm

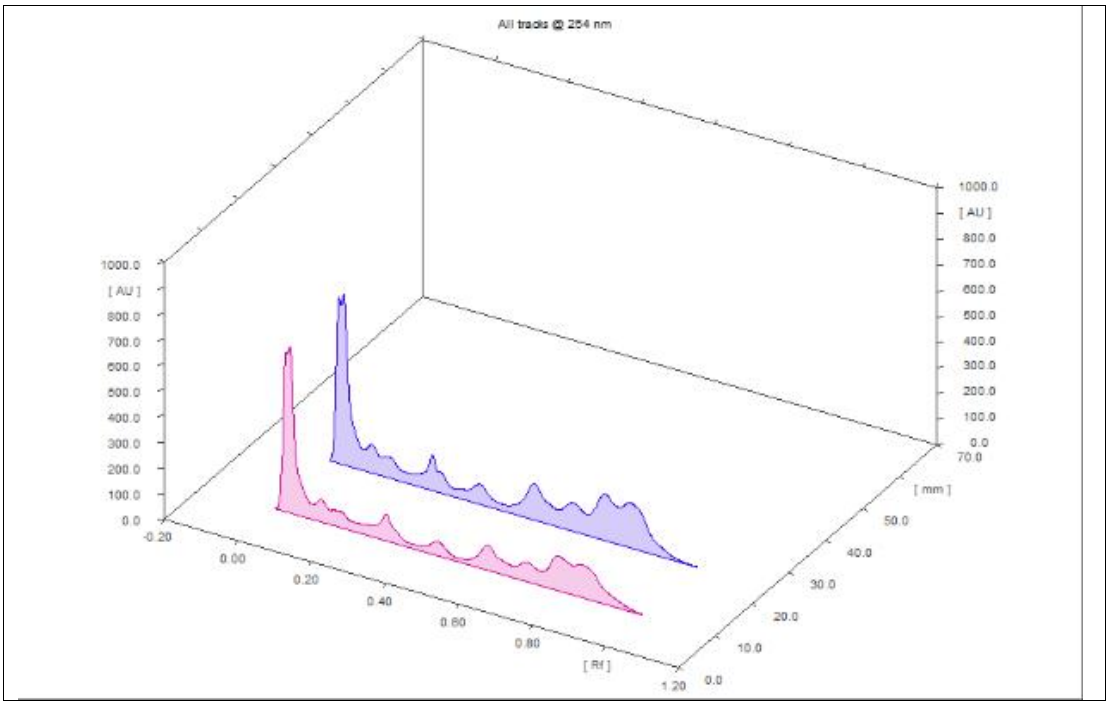
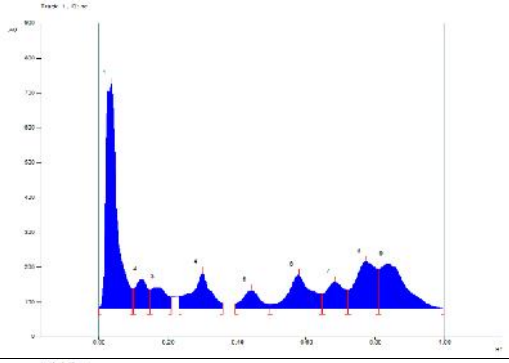
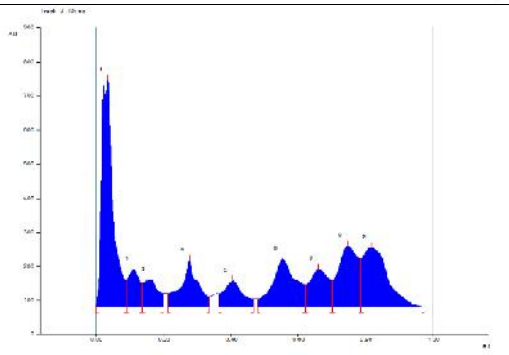


Figure 4: HPTLC finger print profiles and peak tables of Naruvilli chooranam at 254nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.6 AU	0.04 Rf	647.2 AU	46.85 %	0.10 Rf	57.5 AU	14419.3 AU	32.95 %
2	0.10 Rf	57.8 AU	0.13 Rf	85.0 AU	6.16 %	0.15 Rf	54.0 AU	2020.5 AU	4.62 %
3	0.15 Rf	54.0 AU	0.18 Rf	61.3 AU	4.43 %	0.21 Rf	33.7 AU	1968.9 AU	4.50 %
4	0.23 Rf	35.6 AU	0.30 Rf	102.3 AU	7.40 %	0.36 Rf	18.1 AU	3968.3 AU	9.07 %
5	0.40 Rf	15.5 AU	0.44 Rf	52.8 AU	3.82 %	0.50 Rf	11.8 AU	1878.5 AU	4.29 %
6	0.50 Rf	11.9 AU	0.58 Rf	95.6 AU	6.92 %	0.65 Rf	41.8 AU	4420.4 AU	10.10 %
7	0.65 Rf	42.2 AU	0.69 Rf	75.2 AU	5.45 %	0.72 Rf	53.3 AU	2738.7 AU	6.26 %
8	0.72 Rf	53.3 AU	0.77 Rf	134.9 AU	9.77 %	0.81 Rf	13.4 AU	5640.4 AU	12.91 %
9	0.81 Rf	13.4 AU	0.84 Rf	127.2 AU	9.20 %	1.00 Rf	0.3 AU	6698.2 AU	15.31 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	2.4 AU	0.04 Rf	665.9 AU	39.90 %	0.09 Rf	77.6 AU	15957.0 AU	28.59 %
2	0.09 Rf	77.7 AU	0.11 Rf	108.0 AU	6.47 %	0.14 Rf	68.9 AU	2535.0 AU	4.54 %
3	0.14 Rf	68.9 AU	0.16 Rf	79.1 AU	4.74 %	0.20 Rf	38.4 AU	2414.0 AU	4.32 %
4	0.22 Rf	38.8 AU	0.28 Rf	137.5 AU	8.24 %	0.34 Rf	30.0 AU	4778.4 AU	8.56 %
5	0.37 Rf	37.7 AU	0.41 Rf	75.8 AU	4.54 %	0.47 Rf	23.2 AU	2995.9 AU	5.37 %
6	0.48 Rf	23.3 AU	0.56 Rf	140.0 AU	8.39 %	0.62 Rf	64.9 AU	6683.2 AU	11.97 %
7	0.62 Rf	65.1 AU	0.66 Rf	110.2 AU	6.60 %	0.70 Rf	79.0 AU	4332.0 AU	7.76 %
8	0.70 Rf	79.1 AU	0.75 Rf	179.5 AU	10.75 %	0.79 Rf	42.2 AU	7178.6 AU	12.86 %
9	0.79 Rf	142.4 AU	0.82 Rf	173.1 AU	10.37 %	0.98 Rf	0.0 AU	8940.0 AU	16.02 %

Figure 5: HPTLC chromatogram of Naruvilli chooranam at 366nm

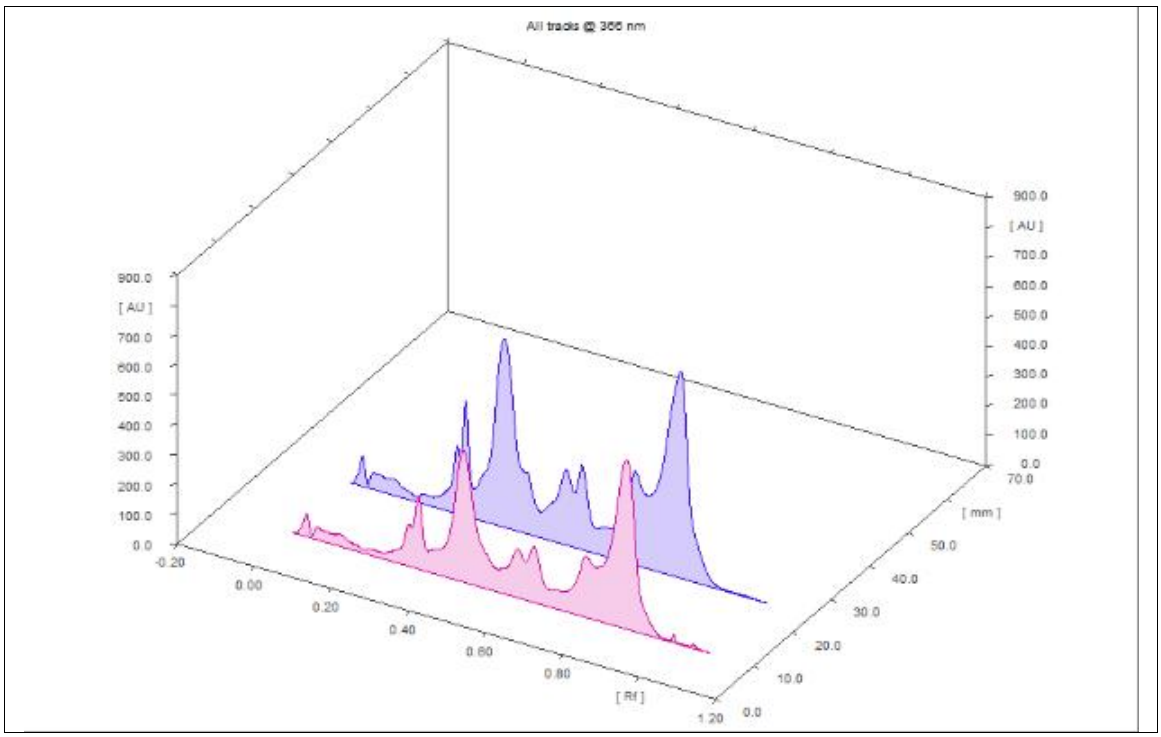
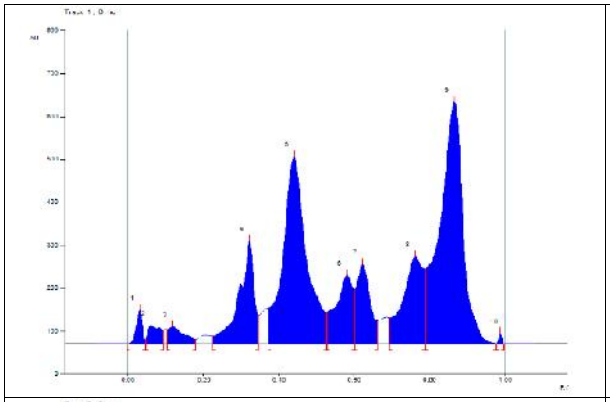
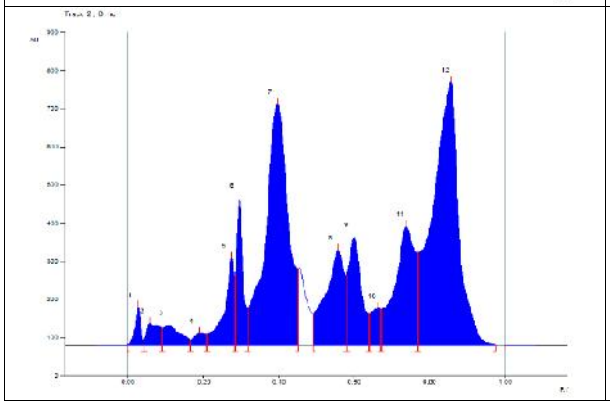


Figure 6: HPTLC finger print profiles and peak tables of Naruvilli chooranam at 366nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.04 Rf	78.8 AU	4.00 %	0.05 Rf	7.7 AU	855.7 AU	1.22 %
2	0.05 Rf	9.9 AU	0.06 Rf	42.1 AU	2.14 %	0.10 Rf	31.0 AU	970.1 AU	1.38 %
3	0.11 Rf	33.0 AU	0.12 Rf	38.7 AU	1.97 %	0.18 Rf	10.0 AU	1171.0 AU	1.67 %
4	0.23 Rf	17.8 AU	0.32 Rf	239.3 AU	12.16 %	0.35 Rf	66.9 AU	6659.2 AU	9.48 %
5	0.37 Rf	81.7 AU	0.44 Rf	436.3 AU	22.16 %	0.53 Rf	73.6 AU	19183.5 AU	27.30 %
6	0.53 Rf	73.9 AU	0.58 Rf	159.8 AU	8.12 %	0.60 Rf	25.8 AU	5054.8 AU	7.19 %
7	0.61 Rf	126.0 AU	0.63 Rf	184.1 AU	9.35 %	0.67 Rf	54.4 AU	4563.4 AU	6.49 %
8	0.70 Rf	60.0 AU	0.76 Rf	203.3 AU	10.33 %	0.79 Rf	74.2 AU	7546.0 AU	10.74 %
9	0.79 Rf	174.3 AU	0.87 Rf	563.0 AU	28.59 %	0.98 Rf	0.3 AU	24152.0 AU	34.37 %
10	0.98 Rf	0.1 AU	0.99 Rf	23.4 AU	1.19 %	1.00 Rf	0.3 AU	116.9 AU	0.17 %



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.4 AU	0.03 Rf	100.3 AU	3.22 %	0.04 Rf	8.3 AU	1036.3 AU	1.02 %
2	0.05 Rf	9.4 AU	0.06 Rf	56.6 AU	1.82 %	0.09 Rf	45.0 AU	1391.0 AU	1.36 %
3	0.09 Rf	45.4 AU	0.11 Rf	52.0 AU	1.67 %	0.17 Rf	15.2 AU	1606.1 AU	1.58 %
4	0.17 Rf	15.4 AU	0.19 Rf	31.2 AU	1.00 %	0.21 Rf	28.4 AU	665.8 AU	0.65 %
5	0.21 Rf	28.6 AU	0.28 Rf	227.3 AU	7.31 %	0.28 Rf	79.9 AU	4133.4 AU	4.05 %
6	0.29 Rf	184.6 AU	0.30 Rf	385.2 AU	12.38 %	0.32 Rf	95.8 AU	4750.8 AU	4.66 %
7	0.32 Rf	96.5 AU	0.40 Rf	632.1 AU	20.32 %	0.46 Rf	90.3 AU	26346.6 AU	25.84 %
8	0.49 Rf	83.0 AU	0.56 Rf	248.3 AU	7.98 %	0.58 Rf	79.7 AU	8722.7 AU	8.55 %
9	0.58 Rf	182.0 AU	0.60 Rf	283.0 AU	9.10 %	0.64 Rf	82.6 AU	6703.7 AU	6.57 %
10	0.64 Rf	82.7 AU	0.67 Rf	95.7 AU	3.08 %	0.67 Rf	95.2 AU	1741.2 AU	1.71 %
11	0.68 Rf	95.1 AU	0.74 Rf	310.3 AU	9.97 %	0.77 Rf	42.4 AU	11863.7 AU	11.63 %
12	0.77 Rf	242.6 AU	0.88 Rf	689.1 AU	22.15 %	0.98 Rf	0.4 AU	33009.2 AU	32.37 %

Figure 6: HPTLC chromatogram of Naruvilli chooranam at 575nm

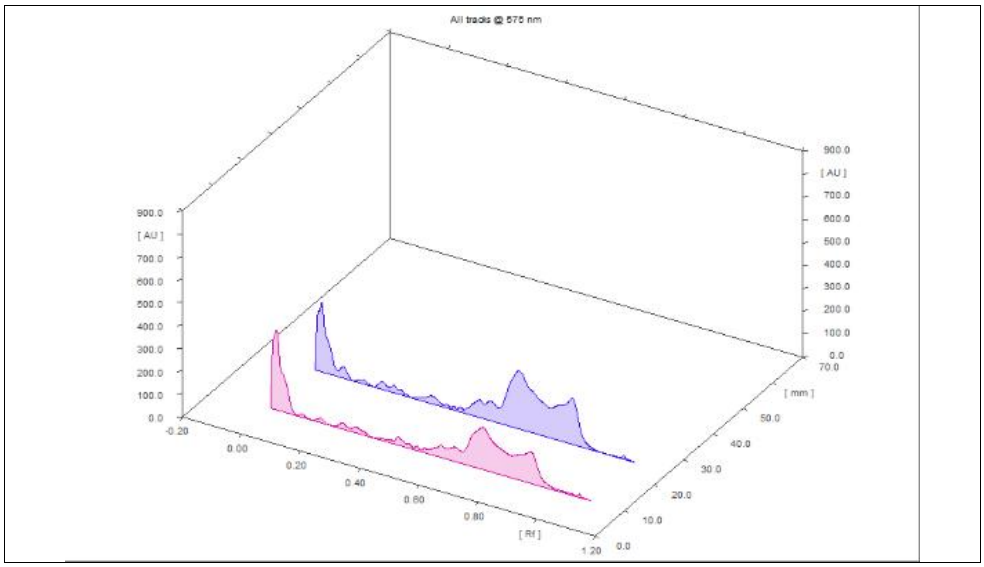
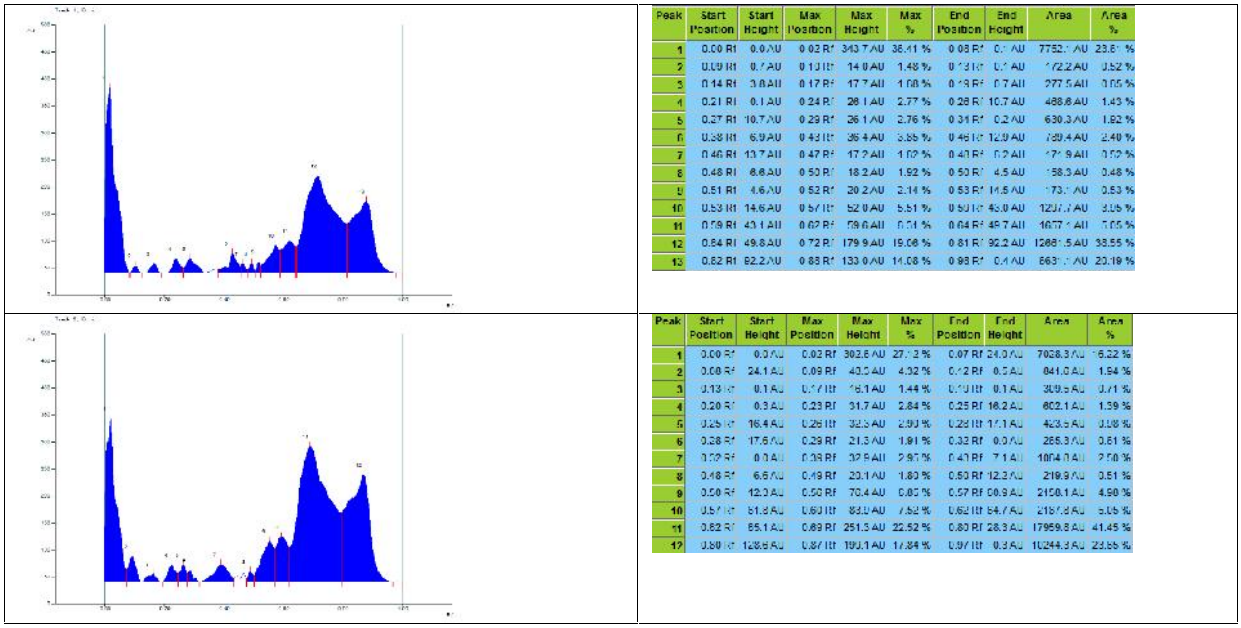


Figure 7: HPTLC finger print profiles and peak tables of Naruvilli chooranam at 575nm



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	343.7 AU	38.41 %	0.08 Rf	0.0 AU	7752.[illegible] AU	23.61 %
2	0.09 Rf	0.7 AU	0.10 Rf	14.0 AU	1.48 %	0.13 Rf	0.7 AU	472.2 AU	0.52 %
3	0.14 Rf	3.0 AU	0.17 Rf	17.7 AU	1.00 %	0.19 Rf	0.7 AU	277.5 AU	0.05 %
4	0.21 Rf	0.1 AU	0.24 Rf	28.1 AU	2.77 %	0.28 Rf	10.7 AU	488.8 AU	1.43 %
5	0.27 Rf	10.7 AU	0.29 Rf	36.1 AU	2.76 %	0.31 Rf	0.2 AU	630.3 AU	1.82 %
6	0.38 Rf	6.9 AU	0.43 Rf	36.4 AU	3.85 %	0.46 Rf	12.9 AU	789.4 AU	2.49 %
7	0.46 Rf	13.7 AU	0.47 Rf	17.2 AU	1.02 %	0.47 Rf	0.2 AU	179.9 AU	0.52 %
8	0.48 Rf	6.6 AU	0.50 Rf	18.2 AU	1.92 %	0.50 Rf	4.5 AU	158.3 AU	0.48 %
9	0.51 Rf	4.6 AU	0.52 Rf	20.2 AU	2.14 %	0.53 Rf	14.5 AU	173.1 AU	0.53 %
10	0.53 Rf	14.6 AU	0.57 Rf	52.0 AU	5.51 %	0.60 Rf	43.0 AU	1207.7 AU	3.05 %
11	0.59 Rf	43.1 AU	0.62 Rf	59.6 AU	6.31 %	0.64 Rf	49.7 AU	1677.1 AU	7.05 %
12	0.64 Rf	49.8 AU	0.72 Rf	179.9 AU	19.06 %	0.81 Rf	92.2 AU	12681.5 AU	38.55 %
13	0.82 Rf	92.2 AU	0.88 Rf	133.0 AU	14.08 %	0.98 Rf	0.4 AU	6631.[illegible] AU	20.19 %

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	302.6 AU	27.[illegible] %	0.07 Rf	24.0 AU	7028.3 AU	16.22 %
2	0.08 Rf	24.1 AU	0.09 Rf	40.0 AU	4.32 %	0.12 Rf	0.5 AU	841.0 AU	1.94 %
3	0.13 Rf	0.1 AU	0.17 Rf	16.1 AU	1.44 %	0.19 Rf	0.1 AU	309.5 AU	0.71 %
4	0.20 Rf	0.3 AU	0.23 Rf	31.7 AU	2.84 %	0.25 Rf	16.2 AU	692.1 AU	1.59 %
5	0.25 Rf	16.4 AU	0.26 Rf	32.5 AU	2.93 %	0.28 Rf	17.1 AU	423.5 AU	0.98 %
6	0.28 Rf	17.6 AU	0.29 Rf	21.3 AU	1.91 %	0.32 Rf	0.0 AU	265.3 AU	0.61 %
7	0.32 Rf	0.0 AU	0.33 Rf	32.5 AU	2.97 %	0.41 Rf	7.1 AU	1084.0 AU	2.50 %
8	0.48 Rf	6.6 AU	0.49 Rf	20.1 AU	1.80 %	0.50 Rf	12.3 AU	219.9 AU	0.51 %
9	0.50 Rf	12.3 AU	0.50 Rf	70.4 AU	6.80 %	0.57 Rf	60.9 AU	2150.1 AU	4.96 %
10	0.57 Rf	61.9 AU	0.60 Rf	83.0 AU	7.52 %	0.62 Rf	64.7 AU	2181.6 AU	5.03 %
11	0.62 Rf	65.1 AU	0.69 Rf	251.5 AU	22.52 %	0.80 Rf	28.3 AU	17959.8 AU	41.45 %
12	0.80 Rf	128.6 AU	0.87 Rf	190.1 AU	17.84 %	0.97 Rf	0.3 AU	10244.3 AU	23.65 %

The TLC profile of Naruvilli chooranam at two different concentrations (1-5 and 2-7 µl) was visualized under UV light (254 and 366nm) and visible light (575 nm), and the result is showed in Figure 1. The selected mobile phase Toluene: Ethyl acetate: Formic acid (5:7:0.1) showed good resolution. The TLC profile of 1-5 µl concentration of Naruvilli chooranam showed 9 spots under UV 254nm (Figure 3) with the maximum Rf value of 0.04, 0.13, 0.18, 0.30, 0.44, 0.58, 0.69, 0.77 and 0.84. The TLC profile of 2-7 µl concentration of Naruvilli chooranam extract showed 9 spots under UV 254nm (Figure 3) with maximum Rf value of 0.02, 0.20, 0.27, 0.36, 0.55, 0.60, 0.75,0.81 and 0.86.

The TLC fingerprint profile of Naruvilli chooranam under UV light at 366nm is showed in Figure 5. The TLC profile of 1-5 µl concentration of Naruvilli chooranam showed 10 spots with the maximum Rf value of 0.04, 0.06, 0.12, 0.32, 0.44, 0.58, 0.63, 0.76, 0.87 and 0.99. The TLC profile of 2-7 µl concentration of Naruvilli chooranam extract showed 11 spots with maximum Rf value of 0.03, 0.06, 0.11, 0.19, 0.28, 0.30, 0.40, 0.60, 0.67, 0.74 and 0.86.

The TLC fingerprint profile of Naruvilli chooranam under visible light at 575nm is showed in Figure 7. The TLC profile of 1-5 µl concentration of Naruvilli chooranam showed 13 spots with the maximum Rf value of 0.02, 0.10, 0.17, 0.24, 0.29, 0.43, 0.47, 0.50, 0.52, 0.57, 0.62, 0.72 and 0.88. The TLC profile of 2-7 µl concentration of Naruvilli chooranam extract showed 12 spots with maximum Rf value of 0.02, 0.09, 0.17, 0.23, 0.26, 0.29, 0.39, 0.49, 0.56, 0.60, 0.69 and 0.87.

Discussion

The TLC profile of (1-5 µl) concentration of Naruvilli chooranam under UV light 254nm showed a spot with Rf value of 0.77 this indicates the presence of phenol Jain *et al*, (2021) reported that quercetin a standard for Phenol showed a spot with Rf of 0.77 under UV light 254 nm using Toluene: Ethyl acetate – Formic acid as mobile phase (3:4:2.5). The TLC profile of (2-7 µl) concentration of Naruvilli chooranam under UV 254nm light showed a spot with Rf value of 0.75 this also indicates the presence of flavonoid Jain *et al*, (2021) reported that catechin a standard for flavonoid showed a spot with Rf of 0.75 under UV light 254 nm using Toluene: Ethyl acetate – Formic acid as mobile phase (3:4:2.5). The TLC profile of (1-5 µl) concentration of Naruvilli chooranam under UV 366nm light showed a spot

with Rf value of 0.32 this indicate the presence of flavonoid Jain *et al*, (2021) reported that rutin a standard for flavonoid showed a spot with Rf of 0.32 under UV light 366 nm using Toluene: Ethyl acetate – Formic acid as mobile phase (3:4:2.5). The TLC profile of (1-5 µl) concentration of Naruvilli chooranam under UV 366nm light showed a spot with Rf value of 0.44 this indicates the presence of flavonoid Amir *et al*, (2013) reported that rutin a standard for flavonoid showed a spot with Rf of 0.44 under UV light using toluene:ethyl acetate:formic acid (5:4:0.5, v/v/v) as mobile phase solvent.

Conclusion

From the above study its concluded that FTIR analysis revealed the potential functional groups and HPTLC analysis showed the versatile phytocomponents such as Rutin, Quercetin, Catechin. Prior studies related to the phytochemicals showed that they are effectively used in the management of Iraippu Noi (Bronchial Asthma). Rf value of the peak ranges from 0.02 to 0.86. It could serves as a valuable primary sources of information for future studies.

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