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HPTLC and FT-IR analysis of Siddha herbal formulation Sivathai choornam

Vasanthi N¹, Komalavalli T²

¹Post Graduate, Department of PG Pothu Maruthuvam, Govt. Siddha Medical College,
Palayamkottai – 627002, Tamil Nadu, India

²Head of the Department, Department of PG Pothu Maruthuvam, Govt. Siddha Medical College,
Palayamkottai – 627002, Tamil Nadu, India

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Abstract

Aim

HPTLC study is to evaluate the phytochemical profiling of Sivathai chooranam and morphology, characterization and biological role are analysed through FTIR spectroscopy.

Materials and methods

The drug is collected and purified as per siddha literature which has been illustrated by Sarabendra Gunmaroga Sigitchai p.no 55 for the management of VATHA GUNMAM (Peptic ulcer disease)

Results

HPTLC - results were obtained in the form of chromatogram at 575nm thus representing the peaks. Further results were described in peak table describing the values of peak area, peak height, percent area and Rf values.

FTIR - The Fourier Transform Infrared Spectroscopy Standardization of Sivathai chooranam is identified 8 peaks contains - Halo compound alkylhalides, alkynes, aromatics, alkenes, sulfoxides,alkene and amides.

Conclusion

From the results and discussion the study revealed the presence of phytochemicals in Sivathai chooranam. The Rf values of peak range from 0.06 to 0.90 in Sivathai chooranam in HPTLC analysis. The study was concluded in the presence of phytochemicals in the siddha polyherbal formulation of Sivathai chooranam which establish the treatment of various diseases.

FTIR standardization of Sivathai chooranam is identified 8 peaks contains. These are the pharmacological actions and therapeutic action of the drug. The presence active components is confirmed the quality, efficacy and therapeutic effect of Sivathai chooranam. This results useful for further research studies related to Sivathai chooranam.

Keywords: Sivathai chooranam, morphology, characterization, HPTLC analysis, FTIR standardization

Introduction

Siddha medicine is one of the oldest medical systems in the world and is very special.

Siddha medicine is very important among the medical systems that originated in Tamil culture. This unique form of Siddha medicine was originated by the Siddhars who lived many centuries ago. Siddha medicine is an excellent medical system based on herbal material, plant material, living material, mineral material. A special medicine has been given from the Sivathai herb to cure stomach ulcers in humans. Sivathai chooranam is very effective in curing stomach ulcers.

Sivathai chooranam was denoted in Siddha literature, Sarabendra Gunmaroga Sigitchai p.no 55 for the treatment of Peptic ulcer disease. The aim of this study is to validate the phytochemical profiling of the siddha polyherbal formulation of Sivathai chooranam through HPTLC study analysis and morphology, characterization and biological role are analysed through FTIR spectroscopy study. HPTLC provides high resolution and accurate data for the phytochemical profiling. HPTLC study was undertaken with CAMAG. FTIR is a suitable technique for analysis of herbal medicine, identification of unknown compounds, quantitative information such as contaminant. It is used to confirm the composition of solids and liquids. HPTLC study results were obtained in the form of chromatogram at 575 nm thus representing the peaks. Further results were described in peak table. FTIR standardization of Sivathai chooranam is identified 8 peaks contains. The presence active components is confirmed the quality, efficacy and therapeutic effect of Sivathai chooranam. This results useful for further research studies related to Sivathai chooranam.

Procedure

HPTLC

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 F₂₅₄ 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 105⁰ 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

Alcohol extract

Solvent system: Toluene: Ethyl acetate: Formic acid (5:2: 0.1)

Volume applied; Track 1- 5 µl: Track 2 – 7 µl

Figure 1: HPTLC profile of Sivathai chooranam

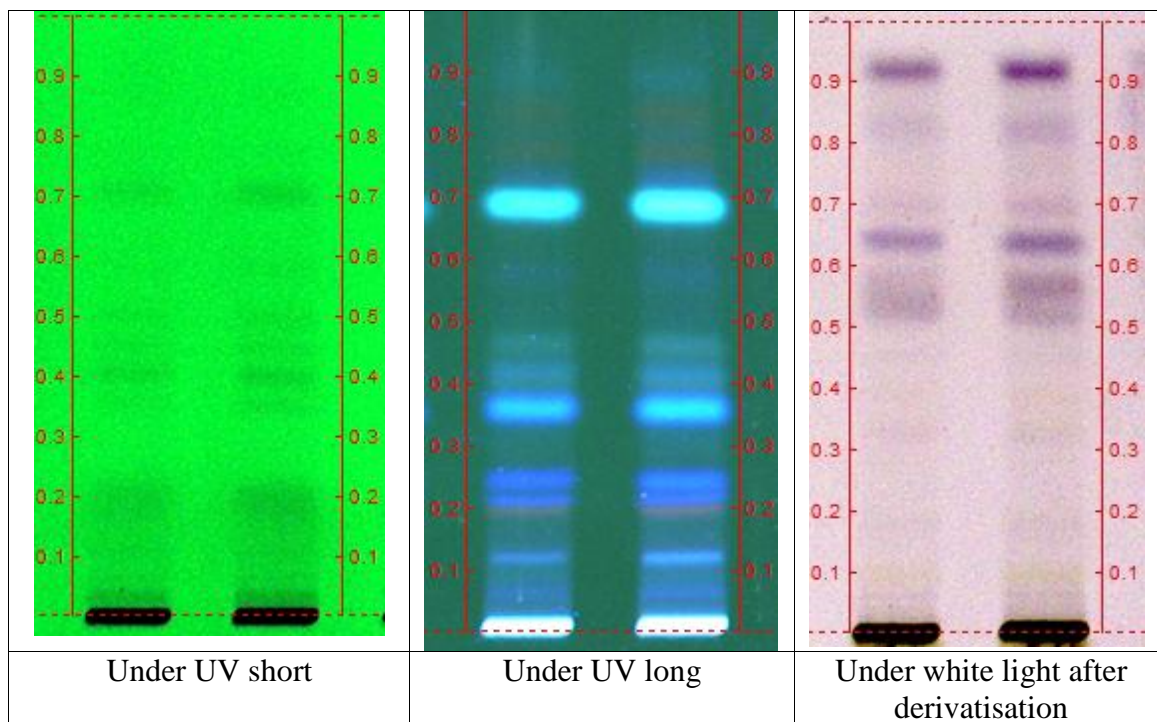


Figure 2: HPTLC chromatogram of Sivathai chooranam at 254nm

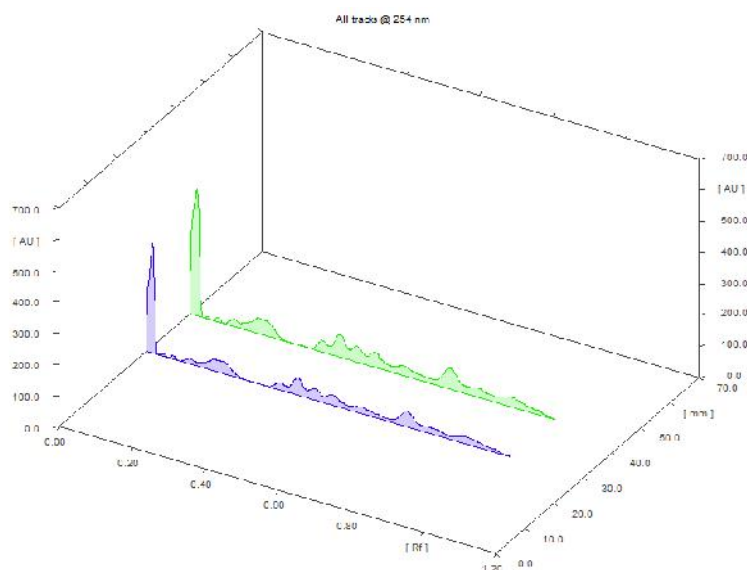


Figure 3: HPTLC finger print profiles and peak tables of Sivathai chooranam at 254nm

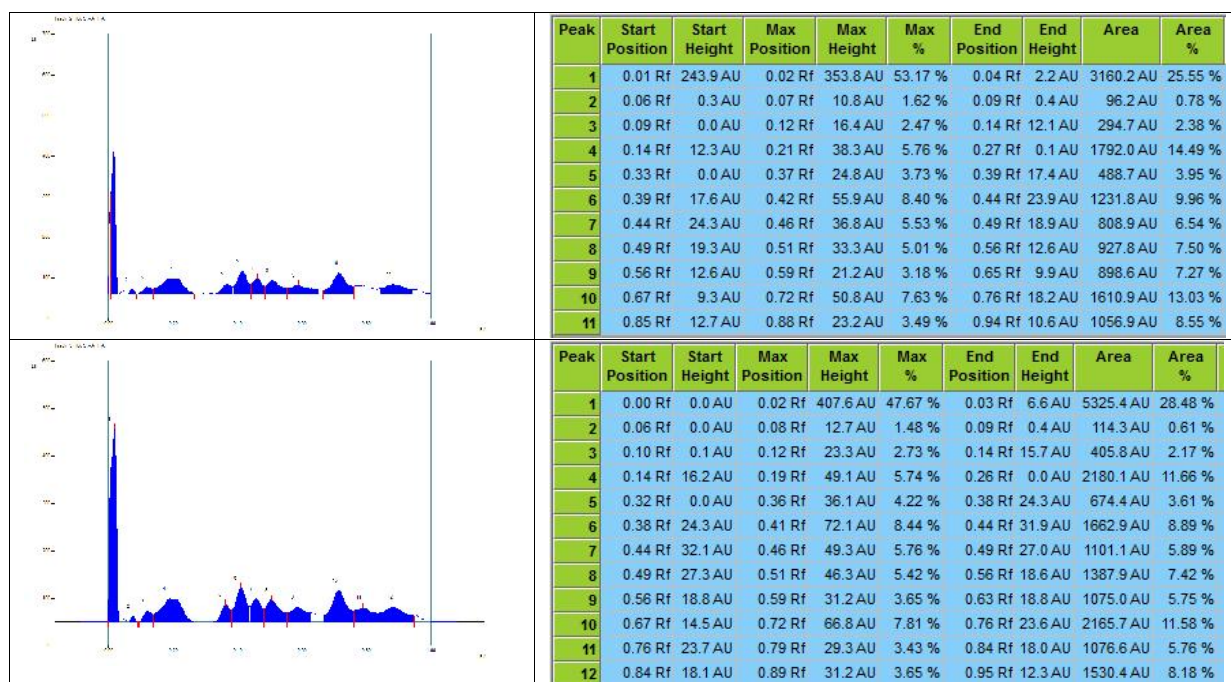


Figure 4: HPTLC chromatogram of Sivathai chooranam at 366nm

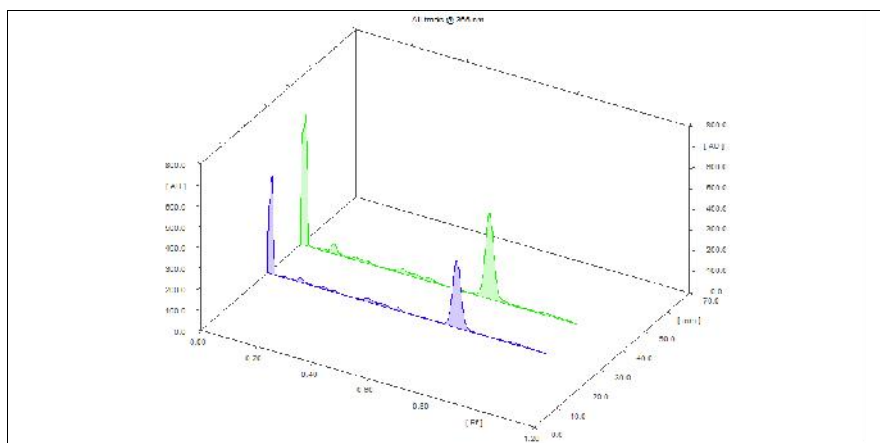


Figure 5: HPTLC finger print profiles and peak tables of Sivathai chooranam at 366nm

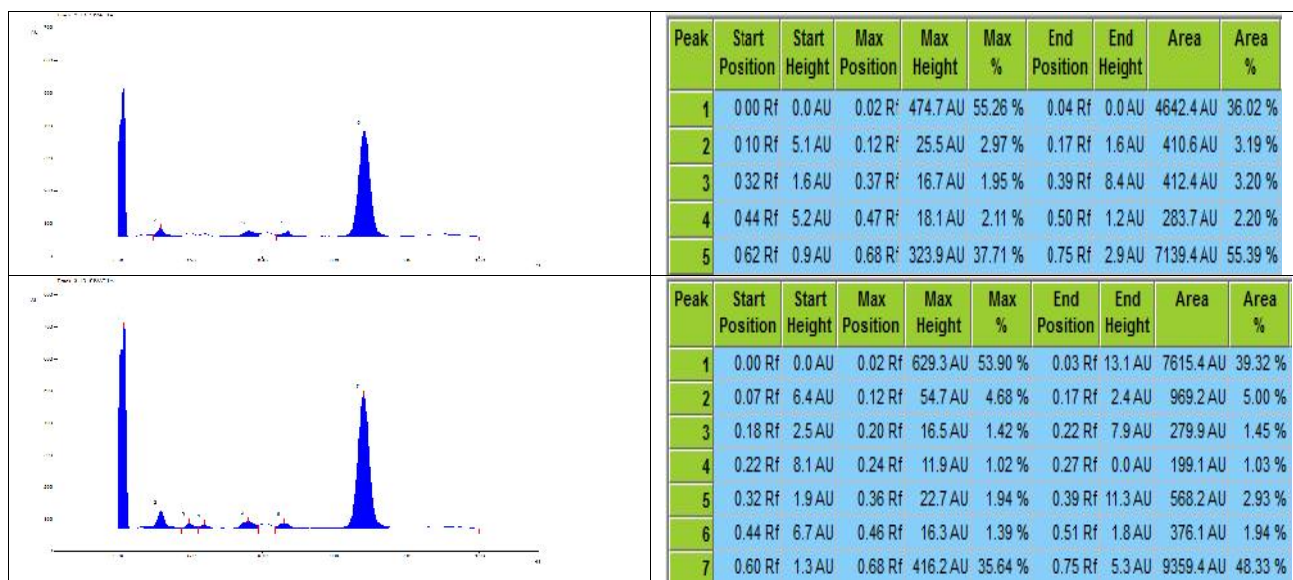


Figure 6: HPTLC chromatogram of Sivathai chooranam at 575nm

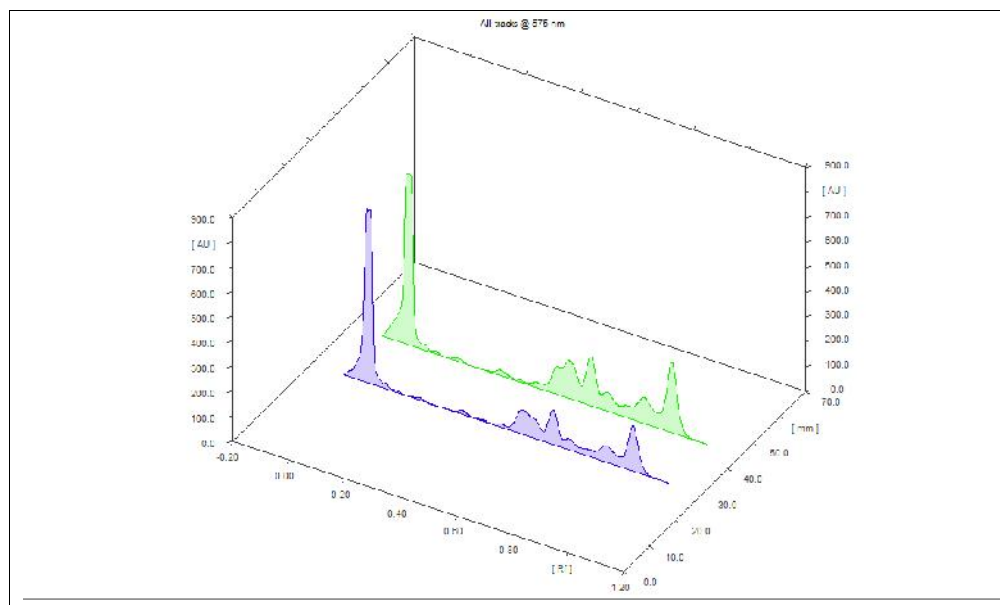
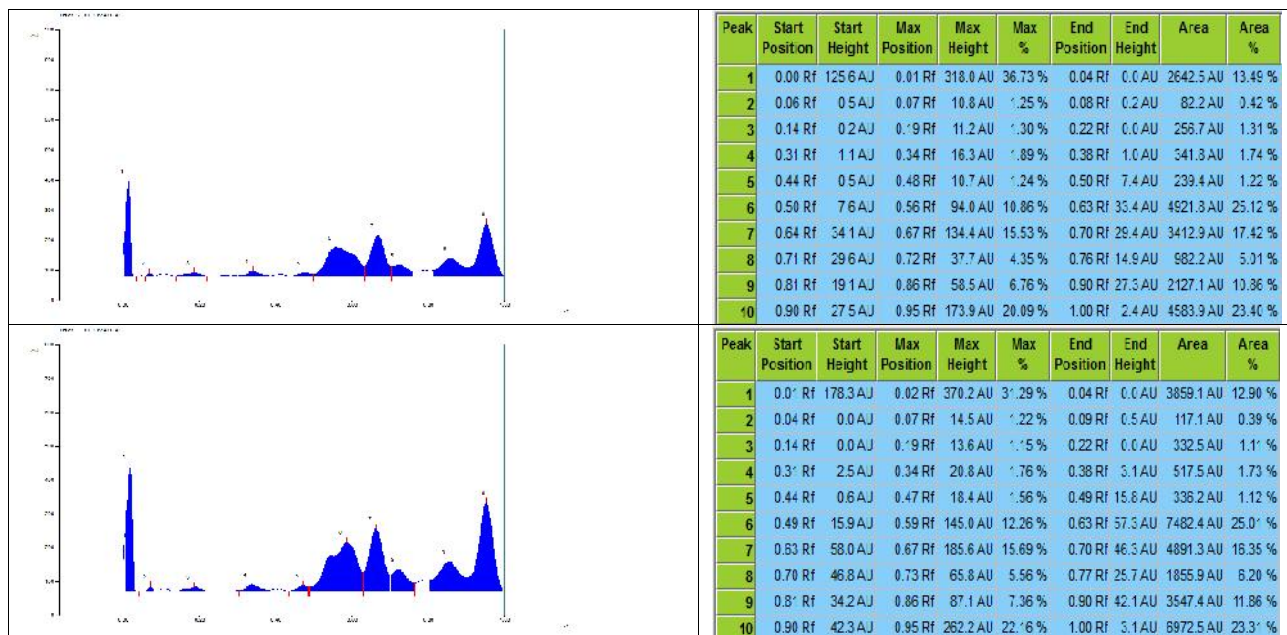


Figure 7: HPTLC finger print profiles and peak tables of Sivathai chooranam at 575nm



Results

The TLC profile of Sivathai chooranam at two different concentrations (1-5 and 2-7 μ l) 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 (5:2:0.1) showed good resolution. The TLC profile of 1-5 μ l concentration of Sivathai chooranam showed 11 spots under UV 254nm (Figure 3) with the maximum Rf value of 0.02, 0.07, 0.12, 0.21, 0.37, 0.42, 0.46, 0.51, 0.59, 0.72 and 0.88. The TLC profile of 2-7 μ l concentration of Sivathai chooranam extract showed 12 spots under UV 254nm (Figure 3) with maximum Rf value of 0.02, 0.08, 0.12, 0.19, 0.36, 0.41, 0.46, 0.51, 0.59, 0.72, 0.79 and 0.89.

The TLC fingerprint profile of Sivathai chooranam under UV light at 366nm is showed in Figure 5. The TLC profile of 1-5 μ l concentration of Sivathai chooranam showed 5 spots with the maximum Rf value of 0.02, 0.12, 0.37, 0.47 and 0.68. The TLC profile of 2-7 μ l concentration of Sivathai chooranam extract showed 7 spots with maximum Rf value of 0.02, 0.12, 0.20, 0.24, 0.36, 0.46 and 0.68.

The TLC fingerprint profile of Sivathai chooranam under visible light at 575nm is showed in Figure 7. The TLC profile of 1-5 μ l concentration of Sivathai chooranam showed 10 spots with the maximum Rf value of 0.01, 0.07, 0.19, 0.34, 0.48, 0.56, 0.67, 0.72, 0.86 and 0.95. The TLC profile of 2-7 μ l concentration of Sivathai chooranam extract showed 10 spots with maximum Rf value of 0.02, 0.07, 0.19, 0.34, 0.47, 0.59, 0.67, 0.73, 0.86 and 0.95.

Discussion

The TLC profile 1-5 and 2-7 μ l concentration of Sivathai chooranam under UV light 254 nm showed a spot with Rf value of 0.46 this indicates this indicate the presence of tannin because gallic acid a standard for phenol under UV light 254nm showed a spot with Rf value of 0.46 using Toluene-ethyl acetate-formic acid methanol (3:3:0.8:0.2) as mobile phase (Banu and Nagarajan, 2014). The TLC profile (1-5 and 2-7 μ l) of Sivathai chooranam under UV light 254 nm showed a spot with Rf value of 0.59 this indicates the presence of phenol Amir *et al*, (2013) reported that quercetin a standard for phenol showed a spot with Rf of 0.44 under UV light using toluene:

ethyl acetate: formic acid (5:4:0.5) as mobile phase solvent. The TLC profile (1-5 μ l) concentration of Sivathai chooranam under UV light 254 nm showed a spot with Rf value of 0.42 this indicates the presence of phenol Baseral *et al*, (2022) reported that gallic acid standard for phenol showed a spot with Rf value 0.42 under UV light 254nm using toluene: ethyl acetate: formic acid (5:4:1) as mobile phase solvent.

II. FTIR Spectroscopy

FT-IR 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.

Figure: 1 FTIR Spectral of Sample Sivathai chooranam

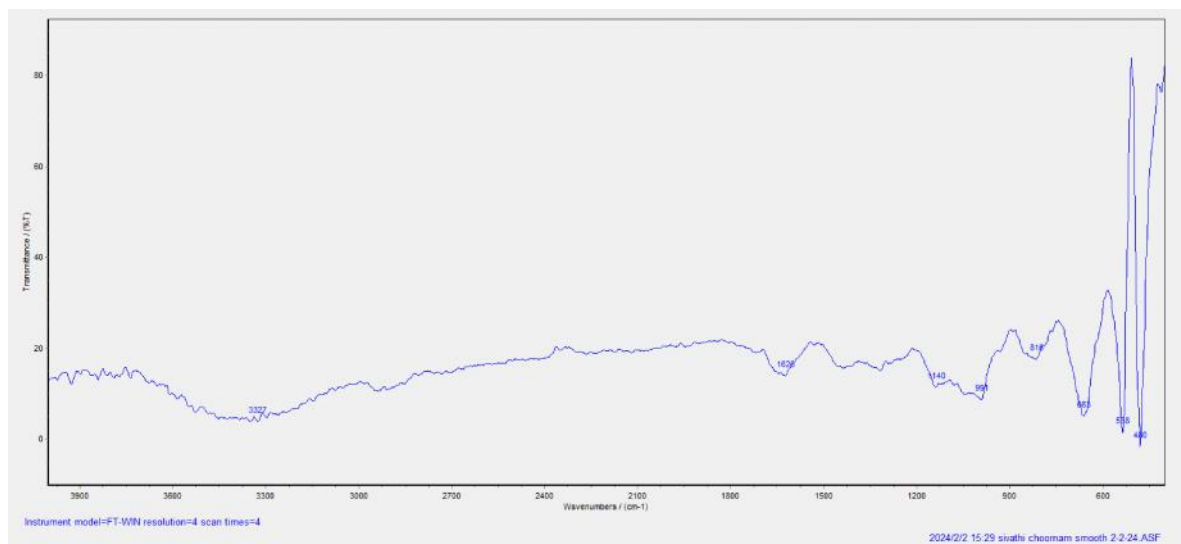


Table: 1. FTIR Spectral of Sivathai chooranam

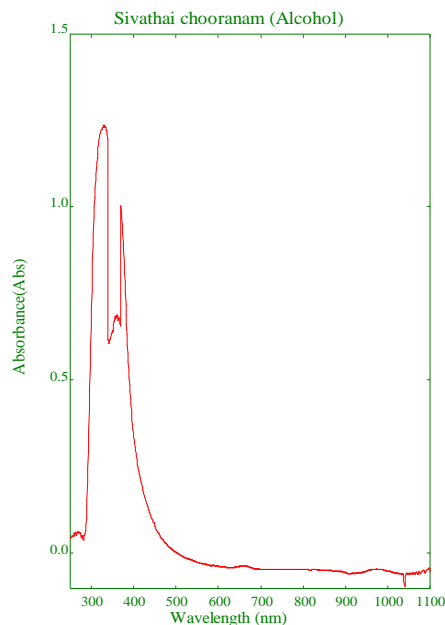
S. No	Peak	Characteristic Absorptions (cm-1)	Possible Functional Group	Class
1	480	300 – 600	C-I stretching	halo compound
2	588	300 – 600	C-Br Stretch	alkyl halides
3	663	600 – 900	C–H bend	alkynes
4	816	600 – 900	C – H out of plane	aromatics
5	991	900 – 1200	=CH out of plane	alkenes
6	1140	900 – 1200	S=O stretching	sulfoxide
7	1628	1500 – 1800	C=C stretching	alkene
8	3327	3300 – 3600	N-H stretching	amides

III. Ultra Violet-Visible (UV-Vis) spectroscopy

The alcohol extract of the drug was subjected to Ultra Violet-Visible spectroscopic analysis. The

extract was scanned at wave length ranging from 200 to 900 nm using UV/VIS spectrophotometer (Model: UV3120) and the characteristic peaks were detected and recorded.

UV- vis Spectroscopy



Conclusion

From the results and discussion the study revealed the presence of phytochemicals in Sivathai chooranam. The Rf values of peak range from 0.06 to 0.90 in Sivathai chooranam in HPTLC analysis. The study was concluded in the presence of phytochemicals in the siddha polyherbal formulation of Sivathai chooranam which establish the treatment of various diseases.

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