

HPTLC ANALYSIS OF SEVVIYADHI CHOORANAM- A SIDDHA POLYHERBAL FORMULATION FOR SINUSITIS

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ABSTRACT

The Siddha system of medicine is one of the oldest unique traditional systems of medicine practiced and followed in South India. It contains many unique formulations for the prevention and treatment of various acute and chronic diseases. The combination of medicines was based on herbal, mineral and Herbo- mineral formulations. However, most of the combinations were herbal based formulations. Among that, one such formulation was Sevviyadhi chooranam, denoted in Siddha literature "Anupava Vaidhya Dheva Ragasiyam- Moondram paagam (Pg no. 466)". It is indicated for the treatment of Sinusitis. The main aim of the present study is to validate the phytochemical profiling of the Siddha polyherbal formulation Sevviyadhi chooranam through High Performance Thin Layer Chromatography (HPTLC) analysis.

Thus HPTLC (High performance thin layer chromatography) is an advance form of TLC (Thin Layer Chromatography) as it provides high resolution and much accurate data for the phytochemical profiling. HPTLC analysis was undertaken with CAMAG TLC Scanner III. The observations and results were obtained in the form of chromatogram at 366 nm thus representing the peaks. The results were described in peak table describing the values of peak area, peak heights, percent area, R_f values.

KEYWORDS: Siddha system, Sevviyadhi chooranam, Phytochemical profiling, HPTLC analysis.

1. INTRODUCTION

Siddha system of medicine is one of the oldest traditional systems of medicine, which has been originated from India and is practiced mostly in the Southern part of this country for treating various diseases including even chronic diseases.^[1] Traditional Medicine System is one of the centuries- old practiced and long serving companions to the human kind to fight against disease and to lead a healthy life.^[2] Sinusitis is the inflammation of sinuses, which are air-filled cavities in the skull. It can be acute or chronic. Types of sinuses are maxillary, frontal, ethmoid and sphenoid. Maxillary sinuses are most commonly affected. Etiology of the sinusitis are both infectious and non-infectious. Infectious etiology includes viral, bacterial and fungi. Non- Infectious etiology includes allergic rhinitis (With either mucosal or polyp obstruction), barotraumas (Deep sea diving or air travel), exposure to chemical irritants.^[3] Its prevalence is about 12.8%.^[4] The HPTLC (High performance thin layer chromatography) is an advanced form of TLC as it provides high resolution and much accurate data, it is accepted all over the world as one of the most powerful analytical techniques used for phytochemical and biomedical analysis.^[5] In Siddha system of medicine there are many distinct formulations were indicated for various diseases among them one such was Sevviyadhi chooranam is indicated for sinusitis as per Siddha literature “Anupava Vaidhya Dheva Ragasiyam- Moondram Paagam”.^[6] The main aim of the current study was to explore the phytochemical profiling of Sevviyadhi chooranam by HPTLC (High performance thin layer chromatography) technique.

2. MATERIALS AND METHODS

2.1 Collection of drugs

The constituents present in the polyherbal formulation was obtained from raw drug store and identified and authenticated by the Botanist, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106.

2.2 Composition of the study drug

Sevviyadhi chooranam, a Siddha polyherbal formulation contains about twelve herbal drugs denoted in Siddha literature “Anupava Vaidhya Dheva Ragasiyam- Moondram Paagam” [6]. The composition is as follows

- | | |
|--|-------|
| 1. <i>Piper nigrum</i> (Black pepper root) | -250g |
| 2. <i>Zingiber officinale</i> (Dried ginger) | -250g |
| 3. <i>Piper longum</i> (Long pepper) | -250g |

4. *Abies spectabilis* (East Himalayan fir) -250g
5. *Cuminum cyminum* (Cumin) -250g
6. *Phyllanthus emblica* (Indian gooseberry) -250g
7. *Plumbago indica* (Indian leadwort) -250g
8. *Cinnamomum verum* (Cinnamon) -250g
9. *Cinnamomum tamala* (Indian bark) -250g
10. *Elettaria cardamomum* (Cardamom) -250g
11. *Piper nigrum* (Black pepper) -250g
12. *Bambusa arundinaceae* (Bamboo salt) -250g

2.3 Purification and Preparation of sample

Purification of drugs

Prior to preparation, all the herbal raw drugs of Sevviyadi chooranam were purified as per Siddha literature “Sikitcha Rathna Deepam Ennum Vaidhya Nool”^[7] as follows

- Black pepper root (Sevviyam)

Purified by peeling out the outer skin and sun dried.

- Dried ginger (Chukku)

A part of dried ginger was treated and bleached with 2 parts of lime stone solution (kal sunnambu) for 3 hours, washed, dried and external scale leaf was peeled off.

- Long pepper (Thippili)

Soaked and treated in leaf juice of *P. indica* for 24 minutes and sun dried.

- East Himalayan Fir (Thalisapathiri)

Purified by washing and sun dried.

- Cumin (Seeragam)

Soil and dust particles were removed and dried in sun light.

- Indian gooseberry (Nellivattal)

Boiled in cow milk and then seeds were removed and sun dried.

- Indian leadwort (Chithiramoolam)

Outer bark was removed, powdered and boiled with steaming method in cow milk and dried.

- Cinnamon (Lavangapattai)

Unwanted dust particles were removed and dried under sunlight.

- Indian bark (Lavangapathiri)

Cleaned and dried under sunlight

- Cardamom (Elakkai)

Unwanted soil and dust were removed and sun dried

- Black pepper (Milagu)

Soaked and treated with buttermilk (sour) for 3 hours and dried under sunlight.

- Bamboo salt (Moongiluppu)

Dissolved in clear water and dried under sunlight to obtain the salt principle.

Preparation of study drug

Sevviyadhi Chooranam, Siddha polyherbal formulation was prepared as per Siddha literature “Anupava Vaidhya Dheva Ragasiyam- Moondram Pagam (Pg.no 466)”^[6]

- After purification, all the ingredients were grounded individually in an iron mortar by using a pestle and sieved with sieving cloth.
- And then all the grounded ingredients were mixed together and stored in an airtight container.

2.4 METHODOLOGY

The study was conducted at Noble Research Solutions, Chennai. The project ID was NRS/AS/0896/09/2022. The parameter used for analysis of phytochemical profiling was HPTLC (High performance thin layer chromatography) analysis. An instrument used for the analysis was CAMAG TLC Scanner III. The TLC plate utilized for the study was Aluminium coated silica gel- Merck. For mobile phase the solvents used were Chloroform: n-Butanol: Methanol: Water: Acetic acid in the ratio of (4:1:1:0.5:0.5). The visualizations of sample drug were observed in chromatogram at 366nm in the form of peaks.

2.5 TLC Analysis

The test sample Sevviyadhi Chooranam was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7 x 6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with ordinary pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro liter by using pipette at distance of 1 cm at five tracks. In the twin through chamber with the specified solvent system. After the run plates are dried and was observed using visible light short- wave UV light 254nm and light long-wave UV light 365 nm.^[8]

2.6 HPTLC (High performance thin layer chromatography) Analysis

High performance thin layer chromatography analysis is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost-effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus, this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprints of phytochemicals which is suitable for confirming the identity and purity of Phytotherapeutics.^[9]

2.7 Chromatogram development

It was carried out in CAMAG Twin Trough chambers. The mobile phase used was Chloroform: n-Butanol: Methanol: Water: Acetic acid in the ratio of 4:1:1:0.5:0.5. Sample elution was carried out according to the adsorption capability of the component to be analyzed. After elution, plates were taken out of the chamber and dried.

2.8 Scanning

Plates were scanned under UV at 366 nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint was developed for the detection of phytoconstituents present in each sample and their respective R_f values were tabulated.

3. RESULTS AND DISCUSSION

The Chromatogram visualization of Sevviyadhi chooranam at 366 nm is shown in fig 3.1. And the 3D chromatogram was shown in fig 3.2. The results of HPTLC fingerprinting were shown in fig 3.3 and the peak table shown in table 3.4 as follows.

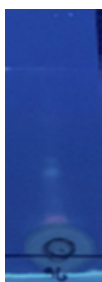


Fig. 3.1: TLC Visualization of Sevviyadhi Chooranam at 366 nm.

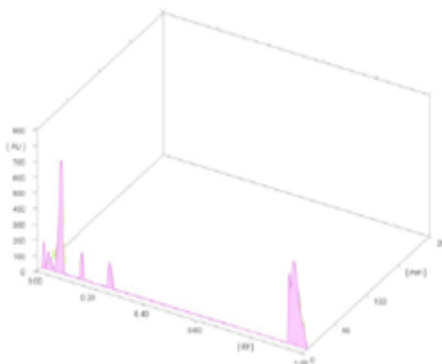


Fig. 3.2: 3D- Chromatogram.

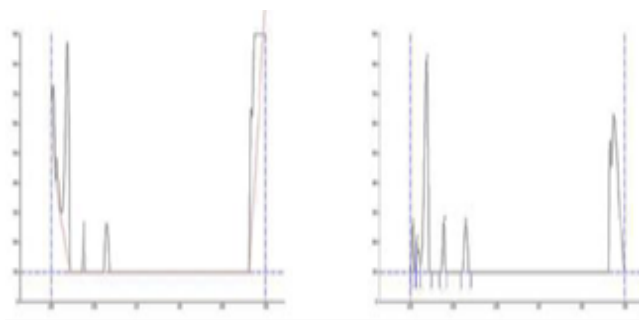


Fig. 3.3: HPTLC Finger printing of sample sevviyadhi chooranam.

Table 3.4 Peak table of sevviyadhi chooranam.

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1	0.00	25.5	0.01	162.1	12.29	0.02	39.4	788.2	6.82
2	0.02	81.7	0.03	106.9	8.10	0.04	14.6	807.5	6.98
3	0.04	14.8	0.07	716.2	54.27	0.10	0.0	7538.0	65.20
4	0.13	0.0	0.15	171.1	12.96	0.17	0.0	941.6	8.14
5	0.24	0.0	0.26	163.3	12.37	0.28	0.0	1486.3	12.86

HPTLC Finger printing analysis of the sample Sevviyadhi chooranam revealed the presence of five prominent peaks corresponds to the presence of five versatile phytochemicals present with it. Rf values of the peaks ranges from 0.02 to 0.24. This validated the phytochemical profiling of Sevviyadhi chooranam helps in treatment of various diseases. Other than this there are many scientific evidences were present about the phytochemicals present in the ingredients of Sevviyadhi chooranam thus increases its therapeutic value for the treatment of various diseases.

Chromatogram GC-MS analysis of methanol extract of *Piper nigrum* showed the presence of fifty-five major peaks and components corresponding to the peaks, thus it contains chemical constituents which many be useful for various herbal formulation as anti-inflammatory,

analgesic, anti-pyretic, cardiac tonic and antiasthmatic.^[10] Twenty-six phytochemicals were found in the hexane extract of *Piper longum*, thus it has high level of bactericidal, anti-tumor, anti-inflammatory, anti-fungal and anti-viral characteristics.^[11] In *Phyllanthus emblica* it contains antimicrobial activity,^{[12][13]} antioxidant activity,^{[14][15]} anti-diabetic activity,^[16] anti-diarrheal activity,^[17] anti-inflammatory activity,^{[18][19]} immunomodulatory activity.^[20] *Plumbago zeylanica* contains alkaloids, steroids, flavonoids, anthraquinones, phlobatinnins, cardiac-glycosides and carbohydrates,^[21] it contains potential therapeutic properties including antiatherogenic, cardiotonic, hepatoprotective, and neuroprotective properties.^[22] Systematic phytochemical investigations on *Abies spectabilis* afforded 72 chemical constituents.^[23] Phytochemicals present in *Cuminum cyminum* are anthraquinone, protein, alkaloid, glycoside, steroid, coumarin, saponin, flavonoid, resin and tannins.^[24] *C. cyminum* seeds consist of many phytochemicals that are recognized to have carminative, anti-flatulent and anti-oxidant properties.^[25] Phytochemical analysis has described important chemical constituents of *Elettaria cardamomum* including carbohydrates, proteins, minerals, lipids, essential oils, flavonoids, terpenoids and carotenoids.^[26] Various parts of *Bambusa arundinaceae* such as leaf, root, shoot and seed possess anti-inflammatory, anti-ulcer, anti-diabetic, anti-oxidant, anthelmintic, astringent and emmenagogue activity.^[27] *Cinnamomum verum* possess great medicinal importance in curing microbial infections, oxidative stress, diabetes, inflammation, wound healing, cancer, anxiety and depression etc.^[28] *Cinnamomum tamala* contains anti-microbial activity.^[29] These scientific evidences further increased and ensured the medicinal value and phytochemical profiling of the Siddha polyherbal formulation Sevviyadhi chooranam.

4. CONCLUSION

From the results and discussion, the study revealed the presence of five versatile phytochemicals in the polyherbal formulation Sevviyadhi chooranam. The R_f value of the peaks ranges from 0.02 to 0.24 in Sevviyadhi chooranam in High performance thin layer chromatography analysis (HPTLC analysis). The present study concluded and validated the presence of phytochemicals in the Siddha polyherbal formulation Sevviyadhi chooranam which establishes its therapeutic potential in the treatment and management of various diseases such as sinusitis.

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