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RESEARCH ARTICLE

High-Performance Thin Layer Chromatography (HPTLC) Profiling of Methanolic Extract of *Sunthi Choorna* (Powder of Dried Rhizome of *Zingiber officinale* Rosc.)

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ABSTRACT

Introduction: High-Performance Thin-Layer Chromatography (HPTLC) is a versatile analytical technique widely employed for qualitative and quantitative analysis across various fields. Known for its high resolution, sensitivity, and reproducibility, HPTLC is particularly effective for separating and identifying complex mixtures. The visual fingerprints generated in chromatograms facilitate quality control, authenticity verification, and compound profiling.

Sunthi, the dried rhizome of *Zingiber officinale* Rosc., is renowned for its therapeutic benefits. Ensuring its authenticity and quality is essential for safe and effective use. Chromatographic fingerprinting provides a reliable method to identify its pharmacologically active and chemically unique components, supporting its validation and standardization.

Materials and Methods: An HPTLC plate measuring 5 × 10 cm, pre-coated with silica gel 60 F254 TLC plates, was used as the stationary phase. Toluene: chloroform: methanol (5:4:1) was used as the mobile phase. Visualization was done under ultraviolet light at 254 nm and 366 nm.

Results and Discussion: The HPTLC of the methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) showed 12 peaks in a total area of 78977.6 AU at 254 nm and 15 peaks with an area of 239963.4 AU at 366 nm.

Conclusion: HPTLC fingerprinting plays a vital role in the standardization of herbal drugs, particularly in the identification of medicinal plants. The fingerprinting profile of *Sunthi Choorna* (powdered dried rhizome of *Zingiber officinale* Rosc.) reveals 12 peaks at 254 nm and 15 peaks at 366 nm. This method provides a detailed profile analysis that is essential for quality control and can be a valuable tool for future research.

1.INTRODUCTION

High-performance thin-layer chromatography (HPTLC) is a highly adaptable analytical technique utilized across numerous fields, including pharmaceuticals, clinical research, biochemistry, cosmetology, food and drug analysis, and environmental studies. What sets HPTLC apart from other chromatographic methods is its ability to present results as visual images. This method offers several key advantages, such as ease of use, affordability, simultaneous analysis of multiple samples, high sample throughput, quick results, and compatibility with various detection techniques. HPTLC excels in separating phytoconstituents and plays a

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crucial role in the verification of raw medicinal substances. Its fingerprinting approach is essential for authenticating herbal drugs, relying on the principle of adsorption to achieve compound separation. Furthermore, HPTLC serves as a valuable tool for quantifying marker compounds, determining purity, and detecting adulteration. This method supports both qualitative and quantitative analysis by identifying specific phytoconstituents through the number of peaks, while the peak size indicates the quantity of each compound present in the sample.

Sunthi is a reputed drug in *Ayurveda*, and it has been mentioned from the *vedic* period to the modern era. It has been cultivated in India for a long time and used as medicine and spice. The useful part of this drug is mentioned as *kandha*. Sunthi has katurasa (pungent taste), laghu (lightness), snigdha (unctuousness), guna (quality), ushna virya (hot potency), and Madhura (sweet) vipaka (post-digestive state) and it is kaphavata samaka (alleviates kapha and vata), deepana (stimulates digestive fire), pachana (aids digestion of undigested food), sophaghna (relieves oedema), vibandhabhedini (relieves constipation), and grahi (absorbs the liquidity of mala) in nighanthus. Sunthi is botanically identified as Zingiber officinale Rosc., belonging to the Zingiberaceae family. Gingerols (6-gingerol, 8-gingerol, 10-gingerol), shogaols (6-shogaol, 8-shogaol, 10-shogaol), paradols, zingerone, zingiberene, β-sesquiphellandrene, curcumene, bisabolene, quercetin, kaempferol, geraniol, polysaccharides, and alkaloids. The phytoconstituents of ginger exhibit diverse therapeutic actions. Gingerols and shogaols provide potent anti-inflammatory, antioxidant, and anti-nausea effects. Zingerone and paradols contribute to antioxidant and anti-cancer activities. Volatile oils like zingiberene, β-sesquiphellandrene, and bisabolene aid digestion, reduce bloating, and possess antimicrobial properties. Flavonoids such as quercetin and kaempferol support cardiovascular health and immunity through their antioxidant effects. Polysaccharides enhance immune modulation, making ginger effective for managing inflammation, digestive issues, and oxidative stress.

The significant therapeutic potential of Sunthi necessitates robust quality control and authentication measures. Chromatographic techniques play a key role in isolating and identifying individual components within herbal drugs by utilizing stationary and mobile phases. A chromatographic fingerprint of an authentic drug serves as a reference to identify active ingredients and helps prevent the use of substandard or adulterated products. This study aimed to develop a comprehensive HPTLC fingerprint of Sunthi Choorna (powder of dried rhizome of Zingiber officinale Rosc.), analyzed under 254 nm and 366 nm for authentication purposes. Such profiling provides a valuable reference for sample identification and verification in future research endeavors.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Drug

The fresh rhizome of *Zingiber officinale* Rosc. was cultivated in the home garden. The harvesting of rhizomes of *Zingiber officinale* Rosc. occurs when the leaves of the plant have turned yellow and the plant has begun to dry up. The plants were carefully forked up, and the rhizomes were expertly taken by cutting off the buds and roots with great attention. The rhizomes have been washed thoroughly to remove any soil residue, using clean water, and soaked overnight in water. The identification of the collected drug specimen was done by the faculty of the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, and plant authentication has been done at St. Albert's
College (Autonomous), Ernakulam, Department of Botany, with a voucher number of 601.

2.2. Preparation of the Dosage form - choorna (Powder)

The outer skin of the fresh rhizome of *Zingiber officinale* Rosc. was removed by carefully scraping it with bamboo splits. After scraping, the rhizomes were kept under sunlight for drying, and frequent turnings were done for uniform drying. After properly drying, they were subjected to powdering. Powdering was done using a pulverizer. The powdered drug was then sieved using a mesh size of 85. The powdered dried rhizome of *Zingiber officinale* Rosc. is shown in Figure 1.

2.3. Preparation of Test Solution

Test solutions were prepared by weighing 2 g of *Sunthi Choorna* (powdered dried rhizome of *Zingiber officinale* Rosc.) and extracting 0.5 g of the sample with 10 mL of methanol. The extract was then filtered to obtain a clear solution for analysis. Thin-layer chromatography was conducted using a mobile phase consisting of toluene, chloroform, and methanol (5:4:1 ratio). A volume of 5 µL of the extract was applied to the HPTLC plate, which was developed up to a distance of 8 cm using the same solvent mixture. Following development, the plate was air-dried and analyzed under ultraviolet light at 254 nm and 366 nm.

2.4. Visualization

The HPTLC plate was observed under UV light at 254 nm and 366 nm. Rf values and color of the resolved bands were recorded.

2.5. HPTLC Conditions

The HPTLC plate used was 5 × 10 cm, pre-coated with silica gel 60 F254 (E. Merck KGaA), with a thickness of 0.2 mm and an aluminum sheet support. The spotting device employed was a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland), using a 100 µL syringe (Hamilton). The developing chamber was a CAMAG glass twin trough chamber (20 × 10 cm), and the densitometer consisted of a CAMAG TLC Scanner 3, linked to WINCATS software.

2.6 Procedure

The HPTLC plate was developed using the specified solvent system in the twin trough chamber, which had been pre-saturated with the solvent for 30 minutes. The syringe was washed twice with methanol before use. The plate was then dried and placed in the scanner. All scanning, integration, and spectrum parameters were configured in the file. For absorption reflection mode, the plate was scanned under UV light at 254 nm and 366 nm, using deuterium, tungsten, and mercury lamps, respectively. All tracks were scanned, and the UV spectrum was recorded. A fingerprint of each track was generated, and the UV spectra of the spots were compared using the spectrum display.

3. RESULTS

3.1. Area and peaks of methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 254nm

maximum Rf value of -0.02 with an area of 22,206.0 AU; maximum Rf value of 0.06 with an area of 6,644.5 AU; maximum Rf value of 0.10 with an area of 2,736.8 AU; maximum Rf value of 0.12 with an area of 4,132.7 AU; maximum Rf value of 0.16 with an area of 3,407.6 AU; maximum Rf value of 0.26 with an area of 9,144.3 AU; maximum Rf value of 0.30 with an area of 4,108.6 AU; maximum Rf value of 0.35 with an area of 3,983.7 AU; maximum Rf value of 0.44 with an area of 2,260.0 AU; maximum Rf value of 0.54 with an area of 16,434.0 AU; maximum Rf value of 0.65 with an area of 2,806.2 AU; and maximum Rf value of 0.73 with an area of 1,113.2 AU. The HPTLC plate view and the overview graph of the methanolic extract of *Sunthi choorna* at 254 nm are illustrated in Figures 2 and 4, respectively. Table 1 presents the peak and area data for the methanolic extract of *Sunthi choorna* at 254 nm.

3.2. Area and peaks of methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 366nm

HPTLC fingerprinting profile of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) showed 15 peaks at the wavelength of 366 nm in a total area of 239,963.4 AU. These 15 peaks were defined at the maximum Rf value of -0.05 with an area of 420.3 AU, a maximum Rf value of -0.01 with an area of 29,804.3 AU, a maximum Rf value of 0.09 with an area of 16,641.3 AU, a maximum Rf value of 0.14 with an area of 19,264.2 AU, a maximum Rf value of 0.18 with an area of 9,537.8 AU, a maximum Rf value of 0.22 with an area of 17,187.4 AU, a maximum Rf value of 0.28 with an area of 11,728.8 AU, a maximum Rf value of 0.31 with an area of 7,507.7 AU, a maximum Rf value of 0.33 with an area of 23,801.7 AU, a maximum Rf value of 0.38 with an area of 7,615.7 AU, a maximum Rf value of 0.42 with an area of 19,878.4 AU, a maximum Rf value of 0.52 with an area of 13,777.5 AU, a maximum Rf value of 0.56 with an area of 26,363.2 AU, a maximum Rf value of 0.60 with an area of 15,775.5 AU, and a maximum Rf value of 0.65 with an area of 20,659.6 AU, respectively. The HPTLC plate view and overview graph of the methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 254 nm is shown in Figures 3 and 5. Table 2 shows the peak and area of the methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 254 nm.

Figure 3: HPTLC plate view of the methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 366 nm

Figure 5: Overview graph of the methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 366 nm

4. DISCUSSION

The present study examines the High-Performance Thin Layer Chromatography (HPTLC) fingerprinting profile of *Sunthi choorna* (the powdered dried rhizome of *Zingiber officinale* Rosc.). HPTLC is a robust analytical technique employed to characterize the chemical composition of herbal formulations by identifying unique chromatographic peaks that signify the presence of distinct phytochemicals. In this investigation, methanol extracts of *Sunthi choorna* were analyzed at two wavelengths, 254 nm and 366 nm, to evaluate their phytochemical diversity and chemical complexity.

At 254 nm, the HPTLC chromatogram exhibited 12 distinct peaks, representing various chemical constituents, with a cumulative area of 78,977.6 AU. The highest peak was recorded at a retention factor (Rf) of -0.02, with an area of 22,206.0 AU. At 366 nm, the HPTLC analysis identified 15 peaks, indicating an even richer phytochemical profile at this wavelength, with a total area of 239,963.4 AU. The most prominent peak at this wavelength occurred at an Rf of -0.01, with an area of 29,804.3 AU. These findings underscore the presence of a wide array of bioactive compounds in the methanol extract of *Sunthi choorna*.

A comparison with a previous study on the hydroalcoholic extract of *Zingiber officinale* revealed 9 peaks at 366 nm, thereby emphasizing how extraction methods and solvents substantially influence the chemical profile of the plant. The greater number of peaks observed in the current study suggests that methanol extraction may be more effective in isolating diverse phytochemicals from *Sunthi choorna*.

Each peak observed in the HPTLC profile corresponds to a distinct chemical component, rendering this technique a valuable tool for quality control and standardization of herbal formulations. The prominent peaks indicate the

presence of major bioactive compounds that may contribute to the therapeutic properties of *Sunthi choorna*.

5. CONCLUSION

The analysis reflects the complex phytochemical composition of *Sunthi Choorna*. Peaks at lower Rf values could correspond to polar compounds, while those at higher Rf values might represent less polar constituents like volatile oils or non-polar metabolites. The prominent peaks suggest the presence of major active constituents. *Sunthi* (dried rhizome of *Zingiber officinale* Rosc.) has a unique pharmacological activity that is attributed to its multitude of active components. For the correct identification of medicinal plants, the HPTLC fingerprinting profile is a crucial component of the standardization of herbal drugs. At 254 nm, it showed 12 peaks in a total area of 78,977.6 AU, and at 366 nm, it shows 15 peaks with an area of 239,963.4 AU. This method is very helpful in identifying adulterations in the herbal drug market.

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7. AUTHORS CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

This study does not require ethical clearance, as it is a laboratory study.

10. CONFLICTS OF INTEREST

Nil

11. DATA AVAIBALITY

This is an original manuscript, and all data are available for review purposes only from principal investigators.

12 .PUBLISHERS NOTE

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Table No: 1 Peak and area of methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 254nm

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.05	-0.02	0.04	22206.0	28.12
2	0.04	0.06	0.09	6644.5	8.41
3	0.09	0.10	0.11	2736.8	3.37
4	0.12	0.12	0.15	4132.7	5.23
5	0.15	0.16	0.19	3407.6	4.31
6	0.22	0.26	0.29	9144.3	11.58
7	0.29	0.30	0.34	4108.6	5.20
8	0.34	0.35	0.41	3983.7	5.04
9	0.42	0.44	0.47	2260.0	2.86
10	0.49	0.54	0.63	16434.0	20.81
11	0.64	0.65	0.69	2806.2	3.55
12	0.71	0.73	0.78	1113.2	1.41

Table No: 2 Peak and area of methanolic extract of *Sunthi choorna* (powder of dried rhizome of *Zingiber officinale* Rosc.) at 366 nm

Peak no.	Start Rf	Max Rf	End Rf	Area (AU)	% Area (AU)
1	-0.05	-0.05	-0.04	420.3	0.18
2	-0.04	-0.01	0.05	29804.3	12.42
3	0.05	0.09	0.10	16641.3	6.93
4	0.12	0.14	0.17	19264.2	8.03
5	0.17	0.18	0.20	9537.8	3.97
6	0.20	0.22	0.26	17187.4	7.16
7	0.26	0.28	0.29	11728.8	4.89
8	0.30	0.31	0.32	7507.7	3.13
9	0.32	0.33	0.38	23801.7	9.92
10	0.38	0.38	0.40	7615.7	3.17
11	0.40	0.42	0.48	19878.4	8.28
12	0.49	0.52	0.53	13777.5	5.74
13	0.54	0.56	0.59	26363.2	10.99
14	0.59	0.60	0.63	15775.5	6.57

Figure 1: *Sunthi choorna* (Powder of *Zingiber officinale* Rosc.)

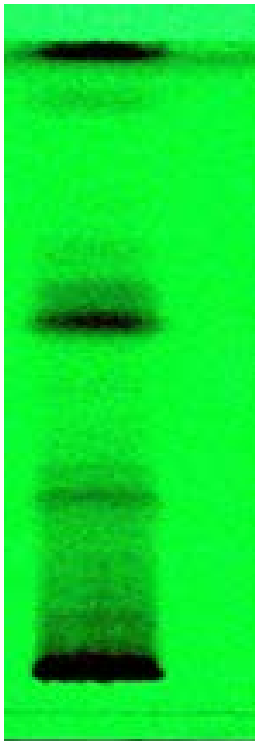

Figure 2: HPTLC plate view of the methanolic extract of *Sunthi choorna* (powder derived from the dried rhizome of *Zingiber officinale* Rosc.) at 254 nm.

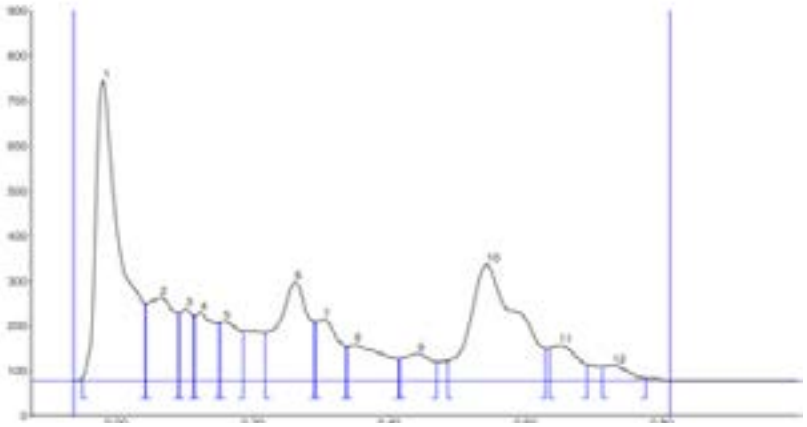

Figure 3: Overview graph of the methanolic extract of *Sunthi choorna* (powder derived from the dried rhizome of *Zingiber officinale* Rosc.) at 254 nm.

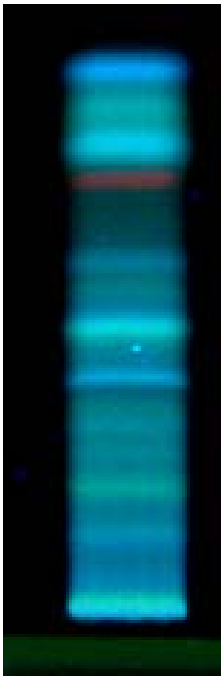

Figure 4: HPTLC plate view of methanolic extract of Sunthi choorna (powder of dried rhizome of Zingiber officinale Rosc.) at 366nm

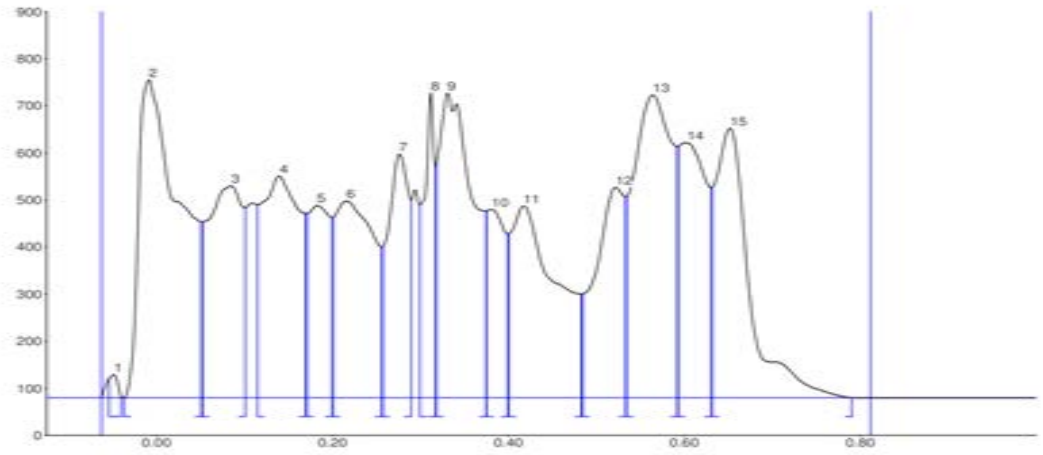

Figure 5: Overview graph of methanolic extract of Sunthi choorna (powder of dried rhizome of Zingiber officinale Rosc.) at 366nm