

## QUALITATIVE ANALYSIS AND QUANTITATIVE DETERMINATION OF “CURCUMIN” IN A SIDDHA HERBO-MINERAL FORMULATION USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

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### ABSTRACT

**Objective:** To perform qualitative and quantitative estimation of ‘Curcumin’ in a Siddha herbo-mineral formulation – ‘SanthaSanthrodyaMathirai (SSM)’ using High-Performance Thin Layer Chromatography (HPTLC). **Method:** Methanolic extracts of three samples of SSM (A, B, C) prepared using the traditional procedures described in Siddha literature. High Performance Thin Layer Chromatography method was performed for quantification of curcumin in all the three samples by employing chloroform: methanol 9.5:0.5 (v/v) as a mobile phase. **Results:** Curcumin was identified at UV- 366 nm using the Retention factor ( $R_f$ ) values in the three samples of SSM with reference to standard curcumin. The percentage of

curcumin present in sample A, B and C is 0.6589, 0.6884 and 0.7104 respectively.

**Conclusion:** Qualitative analysis and quantitative estimation of Curcumin content in SanthaSanthrodyaMathirai (SSM) was successfully done using High-Performance Thin Layer Chromatography (HPTLC). These results suggest that quantification of Curcumin in SSM formulation can be helpful in quality control and the curcumin fingerprints obtained by HPTLC can be helpful in SSM standardization.

**KEYWORDS:** Curcumin, High-Performance Thin Layer Chromatography, Santha Santhrodya Mathirai.

## 1. INTRODUCTION

The Siddha system of medicine, is a gift to the universe and ever since its origin it remains as a precious gem of the medical field due to its in-depth principles covering the physical, psychological, mental and social health of an individual. The pharmacology and pharmacotherapy of Siddha medical system is based on the five elements theory (Panchabootha theory), taste and drug activity based synergism, antagonism (Natpusuvai/pagaisuvai, Natpusarakku/pagaisarakku) in addition to other specific principles.<sup>[1]</sup> Although, the medicinal preparations ascribed in Siddha literature are time-tested standard preparations it is the need of the hour to document standardization procedures based on current analytic techniques to prevent adulteration and to maintain quality control when manufactured in bulk.

Santhasanthrodayamathirai (*SSM*) is an internal drug formulation widely prescribed for hepatic disorders. The ingredients of *SSM* include raw drugs having hepato-protective activity,<sup>[2,3]</sup> and the main ingredient is *Curcuma longa*. *Curcuma longa* is an orange yellow colouring agent. It is a rich source of curcuminoids, curcumin, demoxycurcumin and bis demoxycurcumin which are phenolic compounds that are responsible for the yellow colour of turmeric.<sup>[4,5]</sup> Among them “curcumin” is the principal natural pigment which is widely used in therapeutics.<sup>[6]</sup> “Curcumin” enhances liver detoxification and facilitates the removal of toxins from the body.<sup>[7]</sup> Therefore it is needful to qualitative and quantitatively determine the curcumin content in this traditional preparation *SSM*. Establishment of suitable methods for analyzing and validating plant based formulations for recognition of adulterants and to consistently determine the key bioactive component is a very challenging task for scientists to curtail the batch to batch variation and to access the safety, quality and efficiency of polyherbal formulation.<sup>[8]</sup> Though, there are many analytical methods available for analysis of drugs, we performed High performance Thin layer chromatography (HPTLC) to qualitatively evaluate and quantitatively estimate the curcumin content in *SSM* as this is a simple, efficient, accurate, affordable gold standard method.

## MATERIAL AND METHODS

### 2.1. Preparation of *SSM* drugs

The Study drug *SSM* was prepared by using following raw drugs given in table - 1. Herbal drugs were authenticated by Botanist and minerals were authenticated by Geo-chemist and purified and processed as per the method of preparation mentioned in Siddha literature.<sup>[9]</sup>

**Table. 1: Ingredients of the SSM drug.**

S. No.	Name of the drug	Part used	Quantity
1	<i>Curcuma longa</i> Linn. (Manjal)	Rhizome	300gm
2	<i>Citrus medica</i> Linn. (Lemon)	Juice	As required
3	Mercurous chloride (Pooram)	--	100gm
4	Borax (Vengaram)	--	50gm

## 2.2. Chemicals

Standard Curcumin was purchased from Sigma Aldrich, India and all other chemicals and reagents used in this study were purchased from Merck chemicals, India.

## 2.3. Instruments

CAMAG HPTLC instrument (Automatic TLC sampler, scanner and visualiser, glass twin trough chamber (20cm×10cm×4cm), TLC scanner 3 linked to win Cats software, 0.2 cm thickness pre-coated with silica gel 60F254 (E Merck) aluminium plate were used in this study. Deuterium and Tungsten lamp was used as light source.

## 2.4. Preparation of standard Curcumin solution

Standard Curcumin solution was prepared by dissolving mg of curcumin in 10 ml of methanol corresponding to a concentration of 0.1 mg/ml.

## 2.5. Preparation of sample solutions

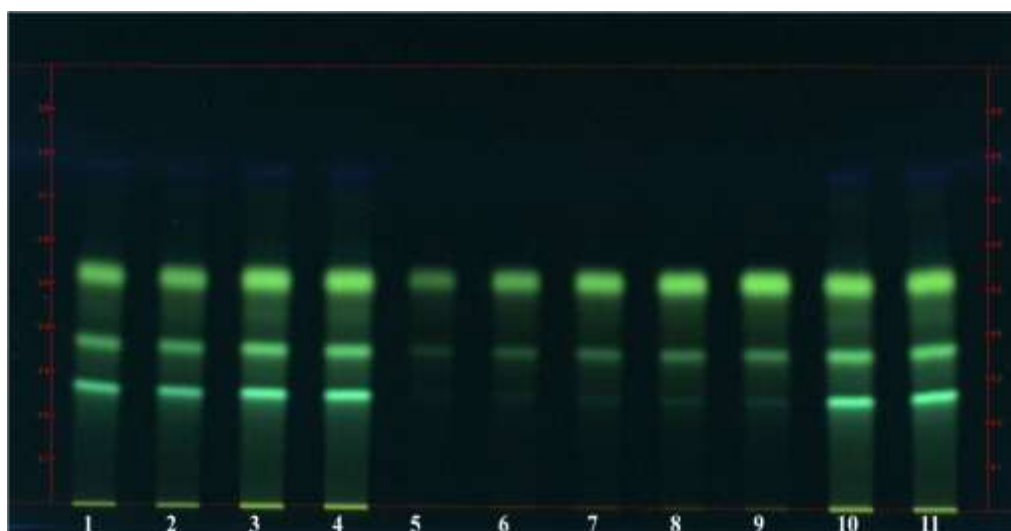
SSM samples A, B and C were weighed accurately and extracted using Soxlet apparatus for 4 hrs using methanol separately (Sample – A= 0.5415 gm, Sample – B = 0.4807 gm, Sample – C= 0.5007 gm). Finally theses extracts were filtered and made up to 25 ml in standard flasks separately.

## 2.6. Development of methods

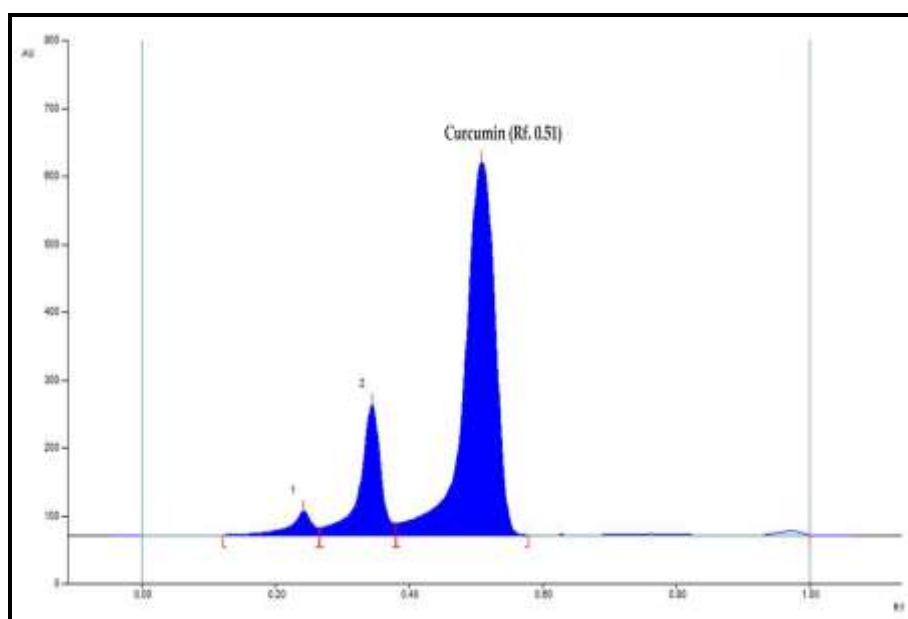
The procedures recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996 was followed. Analysis was performed on 20 cm × 10 cm HPTLC silica gel G60 F254 plates (E Merck) of uniform thickness of 0.2 mm. 3 µl of each sample solutions (A, B, C) in duplicates and 1 µl, 2 µl, 3 µl, 4 µl and 5 µl of standard Curcumin solution (Corresponding to 0.1, 0.2, 0.3, 0.4 and 0.5 µg of the standard per spot) were applied separately. For optimization of method, different mobile phase compositions were employed to achieve good separation. Finally the plate was developed using chloroform : methanol 9.5:0.5 (v/v) as a mobile phase in a twin trough chamber to a distance of 8 cm. The plate was air dried and visualized the plate under UV-366 nm.

### III. RESULTS AND DISCUSSION

The quantitative estimation of biologically active Curcumin phytoconstituents were estimated in the *SSM Siddha* formulation using HPTLC method. Curcumin is the major phyto-constituent present in the formulation and therefore in the quantitative estimation of curcumin was established. Standard Curcumin was showed yellowish green spot at  $R_f$  0.51. TLC plate and HPTLC chromatogram of were shown in figure 1, 2.

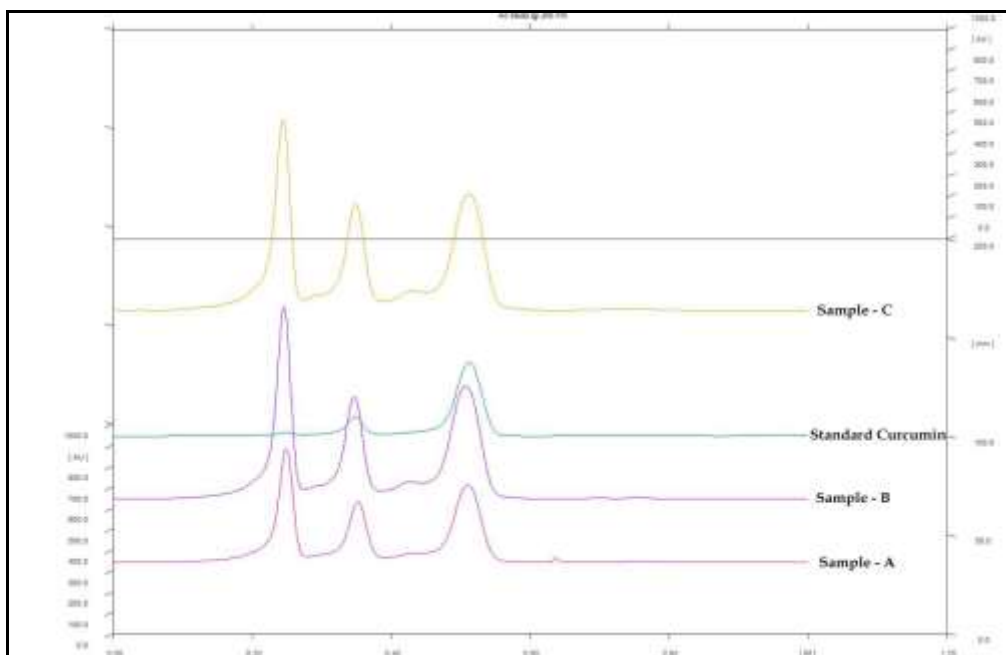


**Fig. 1.** TLC profile of test solutions –A, B and C with Standard Curcumin (Track: 1 and 2 – Sample A; Track 3 and 4 – Sample B; Track 5 to 9 – Standard Curcumin and Track 10 and 11 – Sample C).



**Fig.2.** HPTLC finger print of the standard Curcumin solution

The standard curcumin was present in the three samples of SSM was confirmed by the HPTLC densitometry chromatogram as shown in Fig. 3.

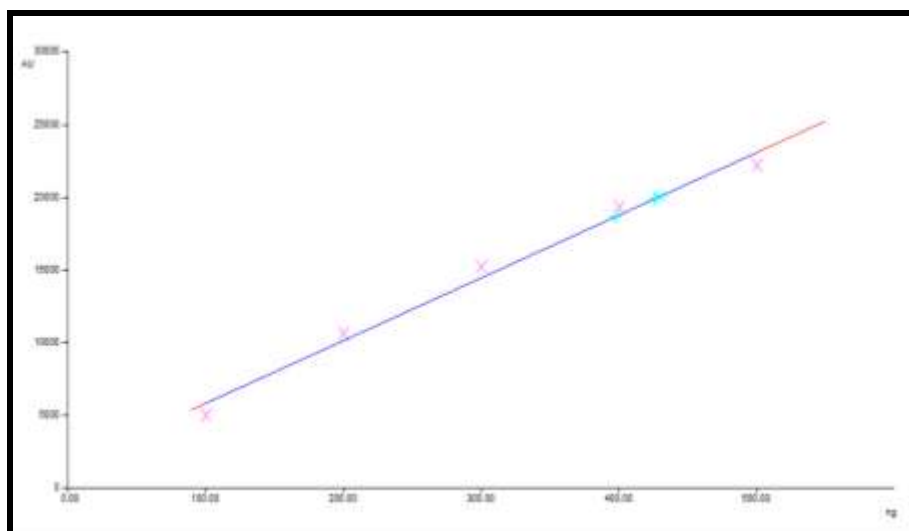


**Fig.3. HPTLC densitometry chromatogram of SSM samples with standard curcumin**

### 3.1. Quantitative estimation of Curcumin in SSM

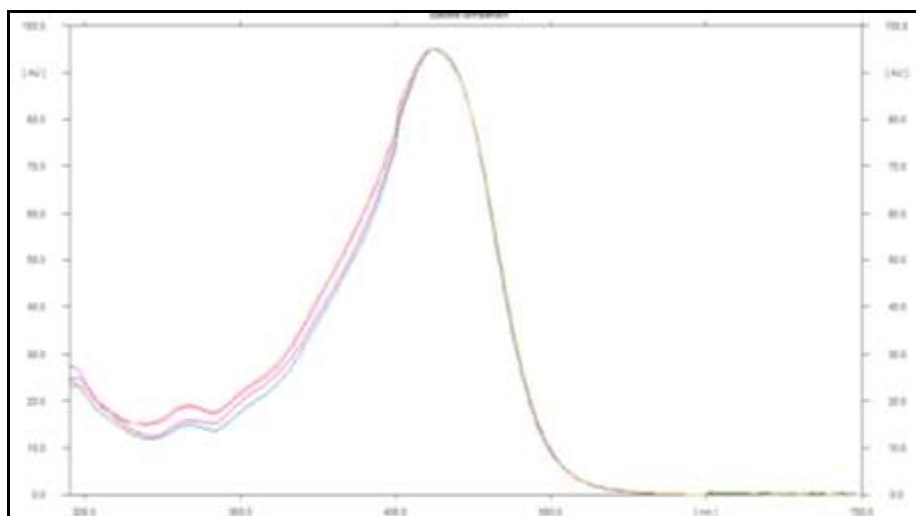
Quantitative estimation of curcumin content in SSM samples were estimated using the calibration curve of standard curcumin.

**Calibration curve:** The respective peak areas of 5 spots corresponding to Standard Curcumin solution were recorded and the calibration curve (Figure 4) was prepared by plotting peak area versus concentration of the Standard Curcumin applied.



**Fig.: 4. Calibration curve of the standard Curcumin with samples of SSM**

The superimposability of UV spectra  $\lambda$  max. at 424 nm (Fig. 5) confirmed the presence of the marker compound curcumin in all three samples of SSM.



**Fig.5. UV-Superimposable spectra curcumin in Sample - A, B, C with curcumin**

### 3.2. Percentage of Curcumin present

The percentage of Curcumin content present in the SSM samples A, B and C was calculated using the calibration curve of Standard Curcumin. The amount of Curcumin present in SSM Sample A is 0.6589 %; SSM Sample B is 0.6884 % and SSM Sample – C is 0.7104 %.

## 1V. DISCUSSION

Raw materials for herbo-mineral preparations are often sourced from various regions and during various seasons. It is for this reason the bioactive content of the raw materials tends to vary significantly and it influences the quality of the medicinal preparation.<sup>[10]</sup> Standardization is a measurement of ensuring the quality control of the drug which is essential for the prevention of adulteration, reproducibility, assessment of finished product, estimation of active principle and global acceptance. Hence, to standardize a herbo-mineral preparation, qualitative and quantitative analysis of the bioactive compound of the major ingredient can be helpful.<sup>[11]</sup>

Curcuma longa, Purified Mercurous chloride, Purified Borax and lemon juice are the ingredients of SSM preparation of which Curcuma longa constitutes the major proportion. Therefore it is essential to quantify the significant bioactive principle which is responsible for its therapeutic efficacy.<sup>[12]</sup> Hence, in this study a validated HPTLC analysis was performed

which determined the presence of “Curcumin” as 0.6589 % in SSM-A, 0.6884 % in SSM-B and 0.7104 % in SSM-C.

Curcumin is a polyphenolic compound derived from turmeric, and is proven to hold miscellaneous pharmacologic effects including anti-inflammatory, anti arthritic<sup>[13]</sup>, anti-ischemic<sup>[14]</sup>, anticancer<sup>[15]</sup>, antioxidant.<sup>[16]</sup> Phase I clinical trials have shown that curcumin is safe even at high doses (12g/day) in humans.<sup>[17]</sup> But due to poor absorption, rapid metabolism and rapid systemic elimination it tends to have poor bioavailability.<sup>[18]</sup> Despite the lower bioavailability, therapeutic efficacy of curcumin against various human diseases including cancer, cardiovascular diseases, diabetes, arthritis, neurological diseases and crohn’s disease, has been documented. The use of adjuvants like piperine, liposomal curcumin, curcumin nanaoparticles, curcumin phospholipid complex and structural analogues of curcumin has been found to improve the bioavailability of curcumin. Enhanced bioavailability of curcumin the near future is likely to bring this promising natural product to the forefront of therapeutic agents for treatment of human disease.<sup>[19]</sup>

HPTLC is a significant tool for qualitative, semiquantitative and quantitative phytochemical analysis of the naturally occurring drugs.<sup>[20]</sup> The present results obtained from this simple and efficient HPTLC method has identified and quantified “curcumin” without any interferences by other compounds (Mercury, borax, citric acid etc.), impurities present in the SSM preparation. And the R<sub>f</sub> value (0.51) corresponding to curcumin was estimated in all the three samples and reference standard was found comparable under UV light at 366 nm. The results of 3 samples also do not significantly differ from each other proving the reproducibility and repeatability of the method. Although, HPTLC method used in this study helped to determine the curcumin content, further studies are required to set the minimum and maximum allowed limits of curcumin in SSM preparation.

## V. CONCLUSION

The hepato-protective activity of the drug SSM is mainly attributed due to the presence of *Curcuma longa* in the preparation making it essential to qualitatively and quantitatively analyze its principal biomarker “curcumin”. The present High performance thin layer chromatography (HPTLC) was performed to ensure the presence of curcumin in significant amounts in SSM preparation and to estimate the percentage of curcumin in SSM. Therefore it can be concluded that High performance thin layer chromatographic technique can be used in routine analysis of qualitative and quantitative determination of curcumin in SSM preparation



and the developed fingerprints of curcumin can be used in quality control and standardization of SSM preparation. With further researches directed towards this, standardization of herbo-mineral drugs like SSM preparation using modern analytical techniques is not a distant thing to achieve.

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## CONFLICTS OF INTEREST

There are no conflicts of interest.

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