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RESEARCH ARTICLE

STANDARDIZATION OF *PANCHA DEEPAKINI CHOORANAM* (PDC)- A SIDDHA POLYHERBAL FORMULATION USING HPTLC FINGERPRINTING

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ABSTRACT

Siddha, one of the ancient Indian systems of Medicine, is a treasure of simple and effective herbal remedies. Contrary to the misconception it accentuates prime importance to herbal formulations than herbo-mineral. *Pancha Deepakini Chooranam* (PDC) is an unique Siddha formulation with five ingredients of botanical origin. Due to its established efficacy in Gastro-intestinal disorders, it is one of the commonly used Siddha formulations. In this study, PDC was prepared in the lab and analysed using HPTLC and it was compared with the selected market sample. Further it was observed for peaks of similar Rf values between the ingredients and final product. This study of HPTLC analysis of various samples show that the traditional formulation can be standardized using such simple and economic analysis techniques.

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INTRODUCTION

Siddha system of medicine classifies diseases into 4448 types. Each of these diseases or disorders is believed to have 64 commonly prescribed types of remedies. Altogether there are more than 3,50,000 herbal formulas used in Siddha system. The Materia Medica of Siddha includes around 4,000 plants, 11 metals, 64 *Pashanam* (mercurial & non-mercurial), 120 *Uparasam* (salts and other minerals) and animal products in preparing medicines. They have classified medicine in to 32 types of internal medicines and 32 external medicines. (Thyagarajan and Siddha materia medica-thaathu vaguppu, 1992) Siddhars also developed the knowledge of bringing down the inorganic substances into atomic and ionic form which are supposed to have increased Bio-availability. In the Siddha system, diagnosis of disease involves identifying its causes, which is done through *Enn vagai thaervu* (Eight fold mode of examination) - examination of *Naadi, Sparisam, Naa, Niram, Mozhi, Vizhi, Malam* and *Moothiram*. The system emphasizes not only on the medical treatment of the diseases but also the patient's environment, meteorological consideration, age, sex, race, habits, mental frame, habitat, diet, appetite, physical condition, physiological constitution etc. (Shanmugavelu *et al*., 2003) Among the 32 types of internal medicines, *Chooranam* is a fine powder of a herbal drug or drugs which is prepared by mixing clean, finely powdered and sieved drugs. Due to divergence in standardization, the medicine prepared using traditional methods may not have the desired quality and batch to batch consistency. Standardization is an essential factor for polyherbal formulations in order to assess the quality of the drugs based on the concentration of their active principles. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical location. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of Siddha medicines so as to ensure quality control and quality assurance. The main problem in poly-herbal formulations is that the presence of each ingredient has to be established. The microscopic character of each ingredient are very difficult to identify and also some time overlapping with the character of the other ingredients. *Pancha Deepakini Chooranam* (PDC) is a widely used Siddha poly-herbal formulation which is therapeutically effective mainly on gastrointestinal disorders. It

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is used as a Carminative, Digestive and Stimulant. (Kuppusamy *et al*., 1998) It is commonly used by Siddha physicians and even as a house-hold medicine. To ensure the efficacy and safety of this formulation, standardization using scientific methods is indispensable. As per WHO guidelines for standardization of herbal drugs, the organoleptic characters, chemical analysis, chromatographic fingerprinting and microbial screening shall be mandatory for herbal formulations. This study is aimed at developing a method to standardize PDC using HPTLC. (Guidelines for the regulation of Herbal Medicines in the South East Asia Region, 2003)

MATERIALS AND METHODS

Sample preparation

The raw drugs were procured from authenticated Siddha pharmacy. The market sample was procured from GMP certified pharmacy. Both the samples were tested at NCNPR (National Centre for Natural Products Research), University of Mississippi, USA. The dried plant materials and commercial sample (each 500 mg) were sonicated with 2.5 ml of methanol for 30 min followed by centrifugation at 3500 rpm for 15 min and then decanted. This was repeated four times and all supernatants were combined, filtered through 0.45 µm filter and the final volume of extracts was adjusted to 10 ml with methanol. (Wagner and Bladt, 2001)

HPTLC plates

Glass plates (Merck, Darmstadt, Germany) with silica gel $60F_{254}$ (10 × 10). Plates were prewashed with methanol and dried in oven. Band width 8 mm, distance between tracks 9.7 mm, first application position 12.0 mm, and application position from the lower edge of the TLC plate is 8.0 mm and dosing rate 90 nl sec^{-1}.

Development

The TLC plates after application were kept in a Twin-trough Chamber saturated using 10 × 10 cm Whatman filter paper for 20 minutes. The TLC was developed using the solvents in the ratio (a) Toluene: Ethyl acetate:: 70:30 and (b) Hexane: Diethyl ether:: 40:60.

List of extracts:

Table 1. The list of extracts

Sample #	Amount	Sample name
1.	10.0 µl	*Zingiber officinale*
2.	10.0 µl	*Piper nigrum*
3.	10.0 µl	*Piper longum*
4.	10.0 µl	Pancha Deepakini Chooranam prepared in Lab
5.	10.0 µl	*Elattaria cardamomum*
6.	10.0 µl	*Cuminum cyminum*
7.	10.0 µl	*Saccharum officinarum*
8.	10.0 µl	Pancha Deepakini Chooranam market sample

Evaluation

The plates after development were removed from the chamber, dried and immersed in Anisaldehyde reagent for 2 sec, and dried in oven for 5 min at 100 °C and images were taken under white light.

Observations:

a. Solvent system - Toluene: Ethyl acetate:: 70:30

The developed TLC plates were observed simultaneously in white light, UV – 254 nm, 366 nm and after derivatisation with anisaldehyde. (Fig.1, 2 & 3). Sample 1 (*Zingiber officinale*) developed three spots corresponding to Rf 3, 5 and 7. Sample 2 (*Piper nigrum*) developed seven spots corresponding to Rf 0.5, 1, 2, 3, 4, 5 and 7. Sample 3 (*Piper longum)* developed six spots corresponding to Rf 0.5, 3, 3.5, 4, 8 and 9. Sample 4 (Pancha Deepakini Chooranam prepared in Lab) developed two spots corresponding to Rf 3 and 5. Sample 5 (*Elattaria cardamomum)* developed one spot corresponding to Rf 3. Sample 6 (*Cuminum cyminum*) developed five spots corresponding to Rf 0.5, 1, 2, 7 and 8. Sample 7 (*Saccharum officinarum*) did not develop any spots in the selected solvent system. Sample 8 (*Pancha Deepakini Chooranam* market sample) developed four spots corresponding to Rf 3, 5, 6 and 9.

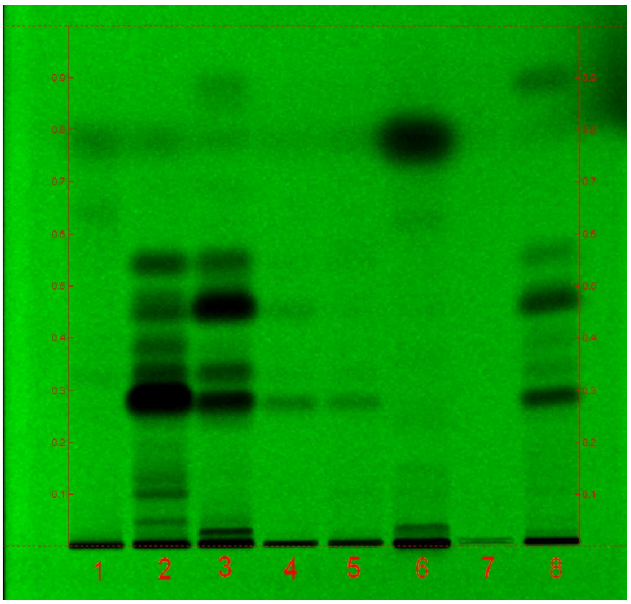

Fig.1. TLC developed with Toluene: Ethyl acetate:: 70:30 Under 254 nm

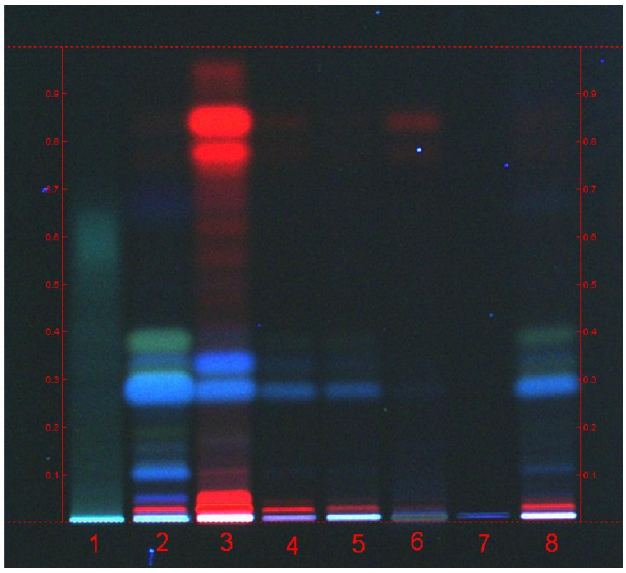

Fig.2. TLC developed with Toluene: Ethyl acetate:: 70:30 Under 366 nm

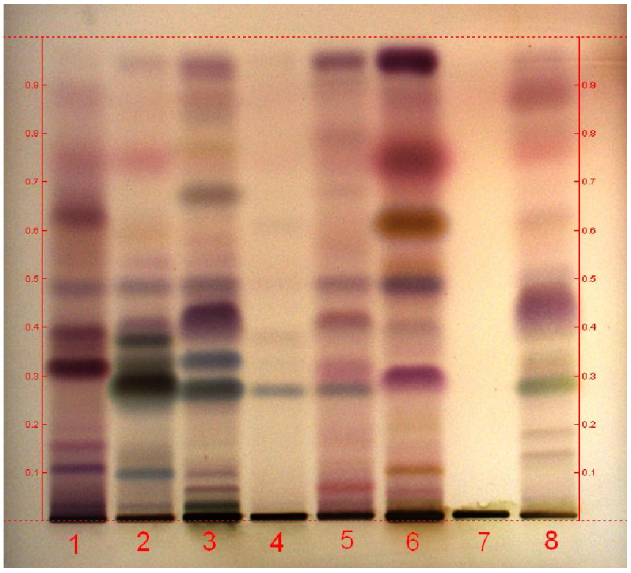

Fig.3. TLC developed with Toluene: Ethyl acetate:: 70:30; derivatisation with Anisaldehyde

b. Solvent system - Hexane: Diethyl ether:: 40:60

The developed TLC plates were observed simultaneously in white light, UV – 254 nm, 366 nm and after derivatisation with anisaldehyde. (Fig. 4, 5 &6)

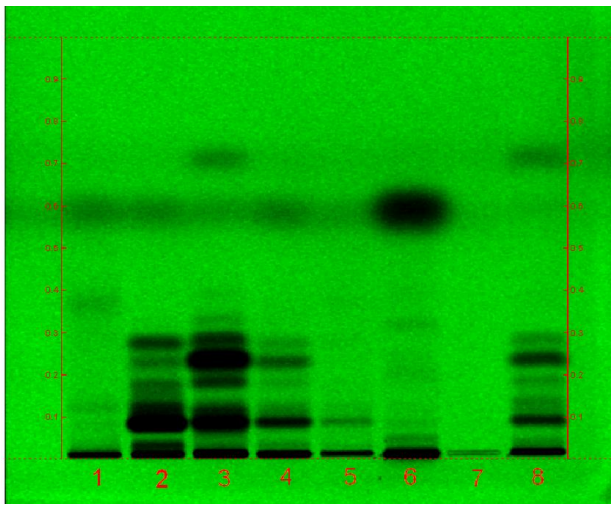

Fig.1. TLC developed with Hexane: Diethyl ether::40:60; Under 254 nm

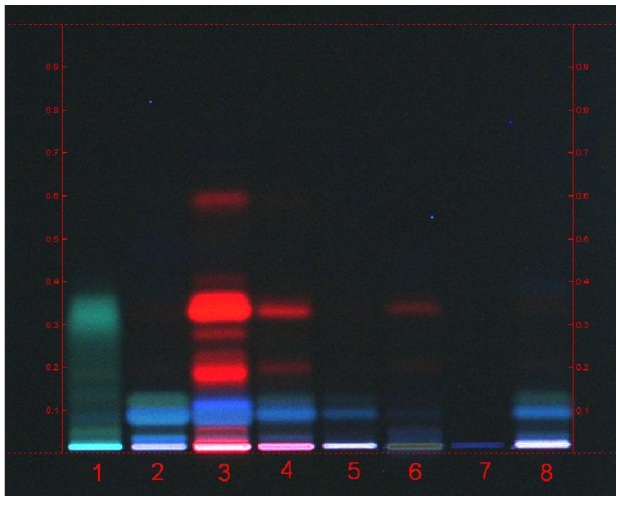

Fig.2. TLC developed with Hexane: Diethyl ether::40:60; Under 366 nm

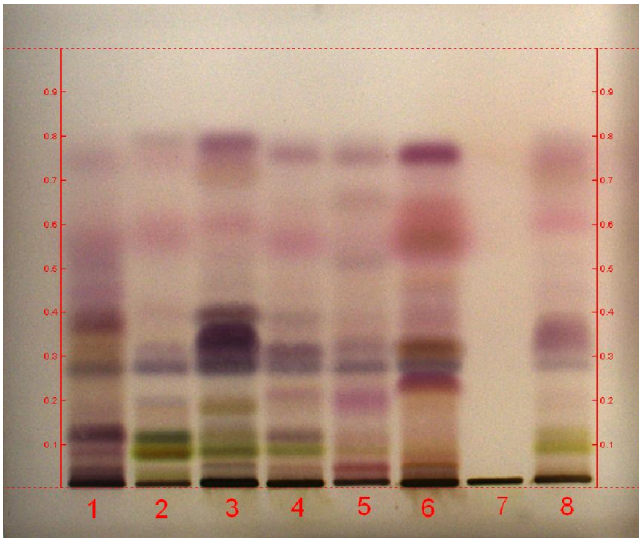

Fig.3. TLC developed with Toluene: Ethyl acetate:: 70:30; derivatisation with anisaldehyde

Sample 1 (*Zingiber officinale*) developed four spots corresponding to Rf 0.5, 1, 2 and 3. Sample 2 (*Piper nigrum*) developed three spots corresponding to Rf 0.5, 1and 2. Sample 3 (*Piper longum)* developed six spots corresponding to Rf 0.5, 1, 2, 3, 4 and 6. Sample 4 (Pancha Deepakini Chooranam prepared in Lab) developed five spots corresponding to Rf 0.5, 1, 1.5, 2 and 3. Sample 5 (*Elattaria cardamomum)* developed two spot corresponding to Rf 1 and 1.5. Sample 6 (*Cuminum cyminum*) developed five spots corresponding to Rf 0.5, 1, 1.5, 2, 3 and 4. Sample 7 (*Saccharum officinarum*) did not develop any spots in the selected solvent system. Sample 8 (*Pancha Deepakini Chooranam* market sample) developed four spots corresponding to Rf 0.5, 1, 1.5, and 4.

RESULTS AND DISCUSSION

a. Solvent system - Toluene: Ethyl acetate:: 70:30

Sample 4 (*Pancha Deepakini Chooranam* prepared in Lab) and Sample 8 (*Pancha Deepakini Chooranam* market sample) were identical with two similar Rf values 3 and 5. Whereas the market sample had additional two spots at Rf 6 and 9 which might be due to the difference in selection of the samples and their maturity. The extracts of *Zingiber officinale, Piper nigrum, Piper longum* along with the lab sample and market sample are showing two spots in common at Rf 3 and 5 which may be due to the nature of alkaloidal compounds common among the ingredients and samples, where as it was not found in *Elattaria cardamomum, Cuminum cyminum, Saccharum officinarum*. Further isolation and characterization of compounds found at Rf 3 and 5 would throw more light on the quantitative methodology of the formulation. This study would be a bench mark in developing a methodology to fingerprint a commonly used traditional Siddha formulation, Pancha Deepakini Chooranam.

b. Solvent system - Hexane: Diethyl ether:: 40:60

The lab sample and market sample were identical with three similar Rf values 0.5, 1 and 1.5. But the lab sample has shown two different spots at Rf 2 and 3 while market sample developed a spot at Rf 4. The spot at Rf 0.5 was identical in samples of *Zingiber officinale, Piper nigrum, Piper longum, Cuminum cyminum,* along with the lab sample and market

sample due to the nature of alkaloidal compounds common among the ingredients and samples, Whereas in *Elattaria cardamomum* and *Saccharum officinarum* it was not observed. The extracts of *Zingiber officinale, Piper nigrum, Piper longum, Cuminum cyminum, Elattaria cardamomum* developed a spot at Rf 1 similar to the lab sample and market sample. This may be due to the nature of alkaloidal compounds common among the ingredients and samples. Further characterization of compounds from Rf 0.5, 1 and 1.5 and isolation would throw more light on the quantitative methodology of the formulation.

Conclusion

Panchadeepakini chooranam, a classical and time tested Siddha formulation is evaluated through fingerprinting techniques using HPTLC and the observations obtained would be an bench mark in developing a standard economic methodology towards developing pharamcopoeial standards for other classical formulations too. Analyzing and comparing the various samples will bring the standards in preparing Siddha formulations. This will serve to maintain the standards in pharma industry.

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