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Authentication of Thiripala Chooranam and mathirai used for *Munnerizhivu* (Impaired glucose tolerance) in siddha medicine

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Abstract

Background: Medicinal plants are playing very active role in traditional medicines for the treatment of various ailments. *Thiripala chooranam* tablet, a polyherbal Siddha formulation is used for treating gastric ulcer, skin disease, constipation, piles, wounds, gum bleeding, liver diseases etc. Therapeutic efficacy of many Siddha formulations depends upon the standardization and authentication of medicinal plants used and hence ensures reproducible quality of herbal products which will lead to safety, quality and efficacy of end products. The drug is being used as a prophylactic and as treatment option in diabetes. However, its effect on prediabetes has not been carried out. The aim of the doctoral study is to evaluate the efficacy of *Thiripala Chooranam* tablet in *Munnerizhivu* (Impaired Glucose Tolerance -IGT).

Objective: To interpret the characteristic parameters of *Thiripala chooranam* tablet to conform its identity, quality and purity.

Methods: *Thiripala chooranam* tablet was evaluated for pharmacognostic, physicochemical and chromatographic parameters.

Results: The pharmacognostic, physico-chemical and chromatographic analysis were found to be appropriate to standardize the *Thiripala chooranam* tablet formulation and can be used as reference standards for the quality control/quality assurance study. The results attained from powdered microscopy serve as a lead for identification of starch grains a poly saccharide which is responsible for management of pre-diabetes (IGT). High performance liquid chromatography analysis showed the presence of important phytoconstituents.

Conclusion: This study provides the scientific data which serves as standard reference for the quality control analysis of *Thiripala chooranam* tablet.

Keywords: *Thiripala chooranam* tablet, starch, polysaccharide, standardization, siddha formulation, pre-diabetes, IGT

Introduction

Siddha system is one among the ancient system of Indian medicine^[1]. This system focuses on leading a healthy life style based on physical, emotional, psychological and social wellbeing. In AYUSH systems, the promotion of preventive approach to achieve the goal of being healthy is attained through holistic treatments.

A pre-diabetic state (IFG, IGT and both) of dysglycemia is defined as IGT which has a strong association with insulin resistance and increased risk of cardiovascular pathology. IGT may precede type 2 diabetes mellitus by many years. It is also a risk factor for mortality^[2]. *Thiripala chooranam* tablet is a potent *Kayakalpam* drug rich in antioxidants and polysaccharides used in the ancient Science of Siddha. It consists of equal parts of the *Terminalia chebula* Retzr., *Embelica officinalis* Gaerth, *Terminalia* and *Terminalia bellerica* Linn. *Kayakalpam* herbs of Siddha possess natural antioxidants which play a vital role in preventing free radical formation and thus preventing NCD's like Pre-diabetes, Diabetes, Hypertension, Cancer and so on. Polysaccharides can have number of potential effects like anti-inflammatory, immune-stimulating, complement activation, anti-thrombotic, antidiabetic and infection protectant activity and many more^[3, 4, 5, 6, 7]. Many medicinal properties are attributed by Thiripala such as anti-aging, antimutagenic, anti-cancerous, anti-inflammatory, antibacterial, antiviral, antioxidant, anti-anemic, antidiabetic, antiparasitic, anti-diarrhoeal, cardioprotective, hepatoprotective, hypocholesterolaemic, radioprotective, colon cleanser and gas distentioner^[8, 9-13]. Hence *Thiripala chooranam* tablet has been studied to validate its efficacy in pre-diabetic state.

Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an

Assurance of quality, efficacy, safety and reproducibility. Standardization ensures the formulation which is ready for package certifies that it contains accurate amount of substance and persuade its therapeutic effects^[14]. WHO has emphasized the need to ensure quality control of medicinal plants products by using modern techniques and by applying suitable standards and parameters^[15].

Henceforth, characterisation of *Thiripala chooranam* tablet was planned to conform its identity, quality and purity through physicochemical, chromatographical and pharmacognostical standardization to ensure the presence of poly saccharides which will be the first step run towards the pre-diabetes.

2. Materials and Methods

2.1 Raw drugs

All the ingredients of *Thiripala chooranam* tablet (Fig.1, Table 1) were procured from the local market of Chennai, Tamil Nadu, India. The collected drugs were identified and authenticated at Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai, Tamil Nadu, India.

2.2 Preparation of *Thiripala chooranam* tablet

The *Thiripala chooranam* was prepared as per the standard method described in pharmacopeia of hospital of Indian medicine. As per the literature, all the ingredients were shade dried and powdered separately, passed through suitable sieve, and then mixed together in required proportions to get uniformly blended *chooranam*. Then the *chooranam* is grinded with *Thiripala kashayam* and made into *Thiripala chooranam* tablet, stored in a clean glass airtight container.

Table 1: Ingredients of *Thiripala chooranam* tablet

Siddha Name	Botanical name	Proportion
Kadukkai	<i>Terminalia chebula</i> Retz.	1 part
Nellikai	<i>Phyllanthus emblica</i> L.	1 part
Thandrikai	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	1 part



Fig 1: Ingredients of *Thiripala chooranam* tablet

2.3 Macroscopy

The organoleptic characters like colour, odour, taste, appearance and texture of *Thiripala chooranam* tablet were evaluated.

2.4 Microscopy

Characters of all the three organized drug ingredients have been observed and recorded. Photomicrographs of diagnostic characters were captured and documented.

2.4.1 Maceration microscopy of ingredients

Cells were isolated by boiling the raw drug pieces in 50% HNO₃^[16]. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axio Cam ERC5s digital camera under bright field light.

2.4.2 Powder microscopy

Microscopic features of powder *Thiripala chooranam* were documented as per standard procedures^[17]. A pinch of the powdered sample was mounted on a microscopic slide with a drop of glycerin-water and the observations were documented. Lignified elements in the *chooranam* were identified after treatment with phloroglucinol and 2 drops of concentrated HCl^[16]. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Nikon COOLPIX 5400 digital camera under bright field light.

2.5 Physicochemical investigation of *Thiripala chooranam* tablet

Physicochemical investigations of *Thiripala chooranam* tablet were carried out using procedures recommended by WHO standard, including determination of alcohol soluble extractives, water soluble extractives, total ash at 600°C, acid insoluble ash, loss on drying at 105°C and pH determinations^[17].

2.6 HPTLC

HPTLC experiments were performed for alcoholic extract of *Thiripala chooranam* tablet on aluminium packed silica gel 60F254 HPTLC plates (Merck). The mobile phase was toluene: ethyl acetate: formic acid (5: 2.5: 0.5) and it was poured into the Camag twin trough glass chamber and allowed to equilibrate for 30 min. The Samples (5 -15 uL) were applied to the plates as sharp bands by using of CAMAG Automatic TLC Sampler 4 (ATS4) applicator. After drying the spots in a current of air the plate was placed in one trough of CAMAG twin trough glass chamber and then developed until the solvent front had travelled a distance of 8 cm above the position of sample application. The developed plate was air dried, visualized under UV 254, 366 nm for documenting the TLC chromatograms; Then scanned in both wavelengths for generating the finger print profiles. The photo documentation and finger printing were also done at 520 nm after dipping the plate in vanillin-sulphuric acid reagent, followed by heating in an oven till the appearance of colour of the spots^[18].

3. Results

3.1 Organoleptic parameters

In organoleptic evaluation, the prepared in-house *Thiripala chooranam* tablet was found to be yellowish brown in colour (Fig. 1.4) with characteristic agreeable odour, slightly coarse texture and tasted astringent and slightly bitter taste.

3.2 Microscopy

3.2.1 Maceration

Maceration microscopy of *Terminalia chebula* fruit showed the presence of pitted parenchyma, fibres and xylem elements showing spiral vessels (Fig. 2); *Emblica officinalis* showed the presence of epicarp cells, parenchyma cells, stone cells and fibres (Fig. 3) and *Terminalia bellerica* showed the presence of stone cells, sclereids, pitted sclereids, fibrosclereids and fibres (Fig. 4). Lignified elements were pitted parenchyma, vessels, stone cells and fibrosclereids (Fig.

5), whereas, mesocarp parenchyma, pitted sclereids, some fibres and vessels were non-lignified (Fig. 6).

3.2.2 Powder

In the powder microscopic analysis of *Thiripala chooranam* tablet, the diagnostic characters such as presence of stone cells and sclereids (*E. officinalis*), stone cells, epidermal cells and epidermal hair (*T. bellerica*) and sclereids, stone cells and fibre with starch grains (*T. chebula*) confirmed the authenticity of the raw drugs (Fig.7).

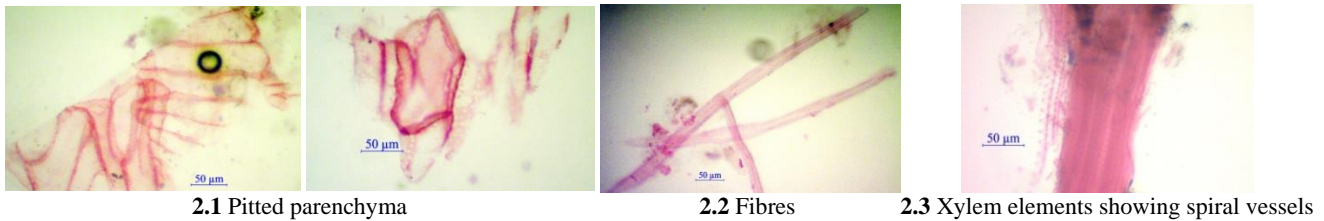


Fig 2: Maceration microscopy of Kadukkai – *Terminalia chebula* fruit rind

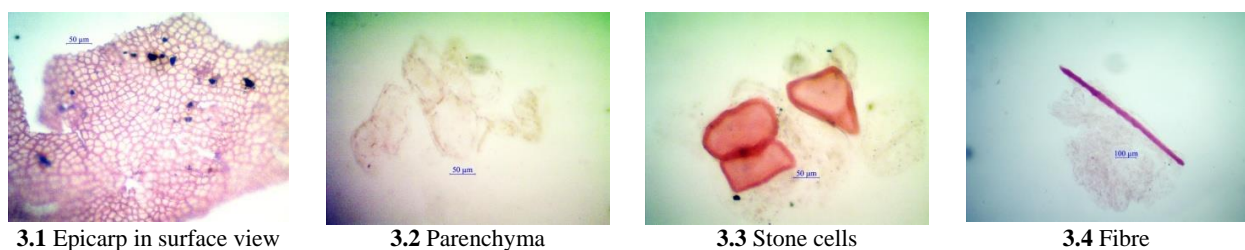


Fig 3: Maceration microscopy of Nellikkai – *Emblica officinalis* fruit rind

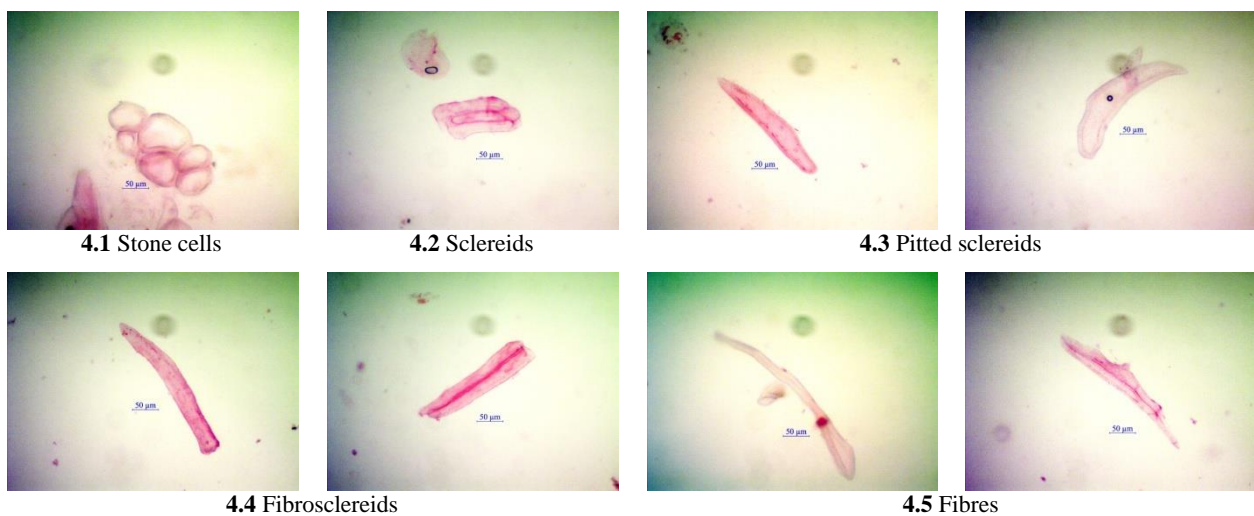


Fig 4: Maceration microscopy of Thandrikai – *Terminalia bellerica* fruit rind

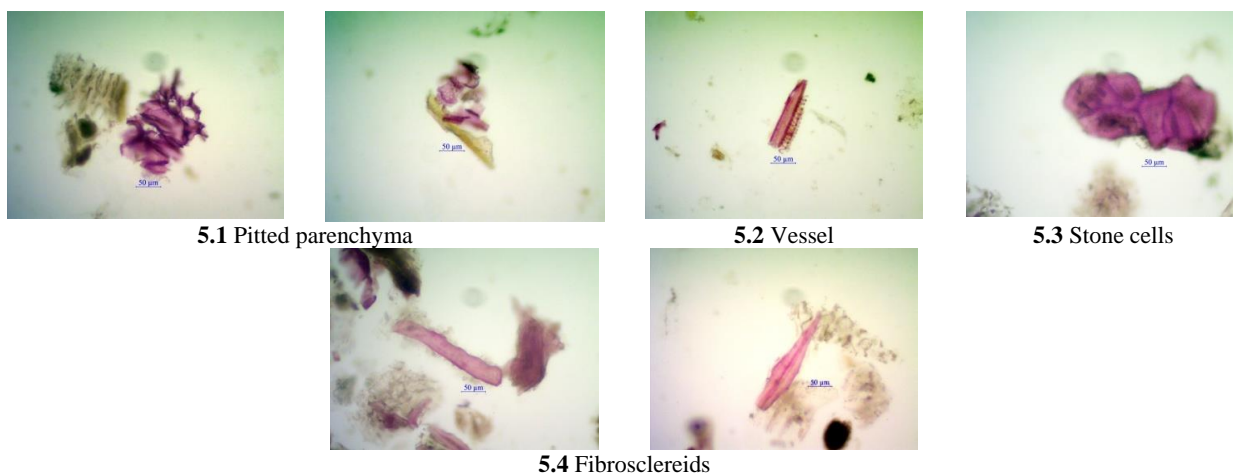
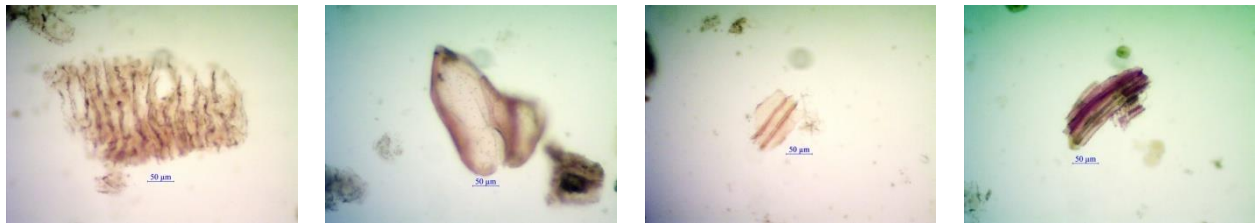


Fig 5: Lignified elements in *Thiripala chooranam*



6.1 Parenchyma of mesocarp 6.2 Pitted sclereid 6.3 Fibre 6.4 Mix of lignified and non-lignified vessel

Fig 6: Non-lignified elements in *Thiripala chooranam*

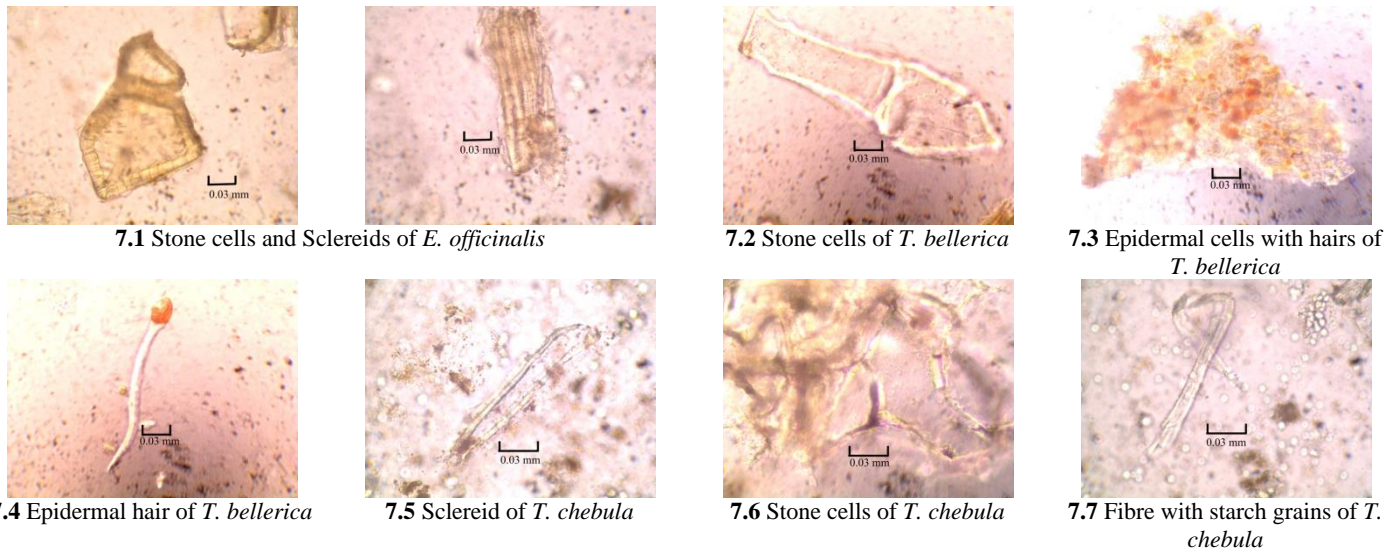


Fig 7: Characters of *Thiripala chooranam* tablet

3.4 Physicochemical investigation of *Thiripala chooranam* tablet

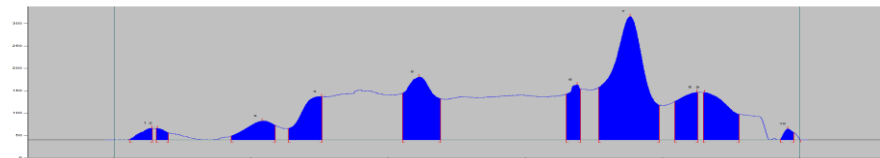
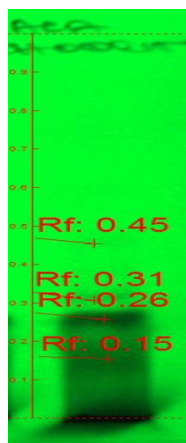
The physico-chemical characteristics *Thiripala chooranam* tablet are presented in Table 2.

Table 2: Physico-chemical characteristics of *Thiripala chooranam* tablet

Parameters	Result
Loss on drying @ 105 °C	4.23 %
Total Ash (@600 °C)	4.27 %
Water soluble ash	2.42 %
Acid insoluble ash	0.62 %
Alcohol soluble extractive	52.93 %
Water soluble extractive	55.95 %
pH	3.36

3.5 Chromatographically analysis of *Thiripala chooranam* tablet HPTLC analysis

Photo documentation of alcoholic extract of *Thiripala chooranam* tablet showed 11, 10 and 13 spots under 254, 366 and white light (after derivatisation) respectively (Figure 3). Densitometric scan at 254 nm revealed 11 peaks corresponding to 11 different compounds in the alcoholic extract, compounds with R_f 0.25 (38.36 %), 0.31 (8.12 %), 0.38 (1.60 %), 0.45 (2.68%), 0.59 (2.16%), 0.74 (1.82%), 0.86 (1.36%), 0.96 (15.04%), 0.97 (12.82%) were the major peaks (Figure 3). At 366 nm there were 10 peaks, peaks with R_f 0.68 (12.71 %), 0.85 (11.01 %), 0.86 (10.98 %) and 0.98 (2.53 %) being the major peaks detected (Figure 4). After derivatisation and visualization of TLC at 520 nm showed 13 bands. Densitometric scan at 520 nm revealed 13 peaks R_f 0.27 (12.80 %), 0.93 (10.97 %), 0.96 (14.71 %) and 0.97 (13.03 %) were the major peaks (Figure 5).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.7 AU	0.01 Rf	12.0 AU	0.99 %	0.03 Rf	6.0 AU	675.3 AU	0.49 %
2	0.08 Rf	0.3 AU	0.16 Rf	181.3 AU	15.04 %	0.16 Rf	80.9 AU	28706.1 AU	20.68 %
3	0.20 Rf	153.2 AU	0.25 Rf	462.3 AU	38.36 %	0.29 Rf	14.8 AU	75011.4 AU	54.03 %
4	0.29 Rf	14.8 AU	0.31 Rf	97.9 AU	8.12 %	0.34 Rf	0.8 AU	8598.8 AU	6.19 %
5	0.35 Rf	0.1 AU	0.38 Rf	19.3 AU	1.60 %	0.39 Rf	4.7 AU	1466.8 AU	1.06 %
6	0.42 Rf	2.1 AU	0.45 Rf	32.3 AU	2.68 %	0.48 Rf	11.4 AU	3933.9 AU	2.83 %
7	0.56 Rf	4.0 AU	0.59 Rf	26.0 AU	2.16 %	0.59 Rf	25.8 AU	1734.0 AU	1.25 %
8	0.71 Rf	12.1 AU	0.74 Rf	21.9 AU	1.82 %	0.77 Rf	13.0 AU	3340.6 AU	2.41 %
9	0.84 Rf	9.3 AU	0.86 Rf	16.4 AU	1.36 %	0.86 Rf	16.3 AU	1052.6 AU	0.76 %
10	0.94 Rf	0.1 AU	0.96 Rf	181.3 AU	15.04 %	0.96 Rf	52.8 AU	8297.4 AU	5.98 %
11	0.96 Rf	152.9 AU	0.97 Rf	154.5 AU	12.82 %	0.99 Rf	1.6 AU	6025.9 AU	4.34 %

8.1 At 254 nm

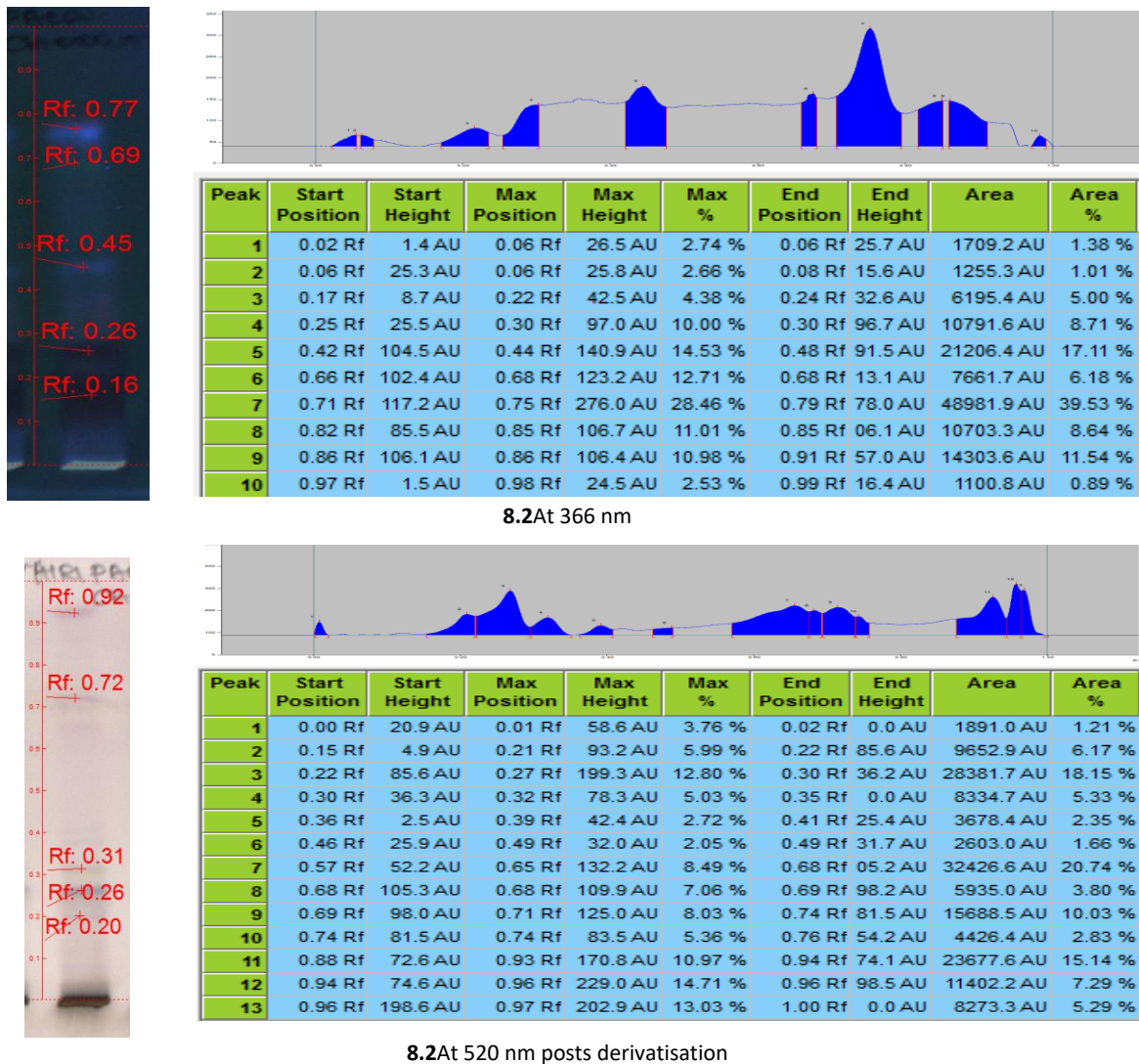


Fig 8: HPTLC fingerprinting of alcoholic extract of *Thiripala chooranam* tablet

Discussion

In the present study the detailed evaluation of *Thiripala chooranam* tablet formulation was assessed for relevant physical, chemical and analytical parameters for its safety and consistent efficacy through quality control measures. Organoleptic properties are the features experienced by the senses, including taste, sight, smell, and touch. Deviation in these properties gives a primary indication about quality variation.

Powder microscopy is used to assess the specific microscopic characters of *Thiripala chooranam* tablet using different staining reagent. These studies provide suitable diagnostic tool for the standardization as well as identification of adulterants. This method is also very useful in confirming presence of ingredients of a polyherbal powder. In our powder microscopic analysis, the microscopic features present in *Thiripala Chooranam* tablet confirmed the presence of all of its herbal ingredients. Microscopy of formulation showed the presence of stone cells and sclereids, epidermal cells with hairs and epidermal cell fragment, sclereid, stone cells. Fibre with bunches of starch grains characteristics of *Terminalia chebula*, *Terminalia bellerica* and *Emblica officinalis*.

In the case of herbal formulations, the presence of moisture content is always unsolicited because it may lead to deterioration of formulation and must be controlled in order to ensure the stability of product. The formulation on analysis for moisture content showed 4.23% w/w (Not more than 15%

w/w) loss on drying which is a good indication for its stability. The total ash value is an indicative of total amount of inorganic material after complete incineration, which might have arisen due to improper washing of individual crude drug involved in formulation manufacturing. The formulation on analysis for total ash yield 4.27% w/w ash which was found to be low and this shows that the formulation is free from adulteration and impurities. Extractive value is another parameter to establish the amount of chemical constituent present in the formulation or to know herb extract ratio. The formulation yields maximum extractive with water, which indicates that water is a potent extracting solvent for the *Thiripala chooranam* tablet formulation. Alcohol also gave appreciable extractive in comparison to water, which indicates that formulation also contains alcohol soluble components. Determination of pH of 1% w/v solution of formulation using a calibrated pH meter showed a 3.36 pH value, which indicates that the plant material yields the acidic solution after extraction with water.

HPTLC is an important tool in standardisation as quality control and fingerprint of herbs of polyherbal formulations can be maintained^[19]. HPTLC has excellent resolution and, therefore, permits simultaneous identification of a wide range of substances in a single run. They also help to identify the individual herbs in herbal formulations. The main objective of the TLC/HPTLC study of *Thiripala Chooranam* tablet was to

develop unique TLC spots in the formulation as identifier of its every ingredient.

The presence of starch in *Thiripala Chooranam* tablet plays a main role in enzymatic action. On hydrolysis it converts polysaccharides into small oligo saccharides and further catalysed by α -glucosidase that converts them into glucose that after absorption enters into the blood stream. Thus, could play a vital role in the management of diabetes²⁰ and pre-diabetes.

Conclusion

The results of present study assure that the formulation conforms with standards and on the measure of quality fits to excellent. *Thiripala Chooranam* tablet was characterized on the basis of the pharmacognostic, physicochemical and chromatographic parameters. The analytical specifications were established for the product with respect to quality based raw materials. The chromatographic data showed presence of all ingredients in the formulation with their unique R_f value. This study may serve as standard reference and the standard operating procedures to be adopted for quality control analysis for future.

Beside the standards, the present study serves as a lead for identification of starch grains which is responsible for management of pre-diabetes.

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