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# Pharmacognostic standardization and development of HPTLC fingerprint fora Siddha herbal formulation Injiirasayanam

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**Abstract**
The present study evaluates the pharma cognsotic standardization of a Siddha herbal formulation, Injiirasayanam based on its botanical and physico-chemical parameters, which ensure the quality and safety of formulation from adulterants. Powder microscopical studies of formulation and anatomical studies of individual ingredients were carried out. Physico-chemical parameters such as loss on drying at 105 $^0$C, total ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive were also determined. High performance thin layer chromatographic study (HPTLC) of Injiirasayanam (HPTLC) was performed and the chromatograms were documented. Physico-chemical standards of the formulation were established for the safety and quality evaluation of this formulation. High performance thin layer chromatography (HPTLC) is a valuable tool for the investigation of herbal products with respect to different aspects of their quality. Thus, physico-chemical standardisation and HPTLC fingerprinting helped to ensure the quality of formulation and to identify the presence of phyto components based on its $R_f$ values.

**Keywords:** Injiirasayanam, *Zingiber officinale*, *Cuminum cyminum*, pharmacognostic, HPTLC

## 1. Introduction
Injiirasayanam is a herbal formulation used in Siddha system of medicine against various ailments. The ingredients of the formulation are *Zingiber officinale*, *Cuminum cyminum* and ghee. The formulation named as Injiirasayanam in Siddha Formulary of India (Part-I) is being mentioned as Injichooranam in the reference text Siddha Vaidhya Thirattu. The main therapeutic usages are for gastric problems, acidity, constipation, stomach pain, vomiting etc. Nowadays, raw drugs are getting mixed with both substitutes and adulterants. Hence, it is mandatory to ensure the quality control of drugs. Scientific validation of safety and efficacy of drugs before going to administer in humans are essential [1].

Basically the formulations Chooranam & Irasayanam are defined in Siddha Formulary of India as follows 1) Chooranamis the fine powder of formatted raw drug(s) mixed up well in exact ratio for homogeneity. The prepared Chooranam should be devoid of moisture content and tackiness. 2) Irasayanam is prepared by the process of mixing certain powdered raw drug(s) with Jaggery, ghee and smashing into the substance of loose or thick consistency.

Event hough the processing method of Injiirasayanam is inappropriate with the SOP of Irasayanam, the ingredients mentioned - Ney and Charkkarai are reliable for the preparation of Irasayanam only and not for the Chooranam. Hence, the Siddha Pharma copoeial Committee has classified this preparation as an Irasayanam.

*Z. officinale* belongs to Zingiberaceae is well known for its aroma and therapeutic properties. The main medicinal part, the rhizome is aromatic and used as anti-emetic and carminative. It is having cardiovascular and platelet aggregation property [2]. *C. cyminum* (Apiaceae) is a plant used as a drug in Ayurveda and Siddha system of medicine as it possesses carminative, antibacterial, antioxidant and antidiabetic properties [3]. The medicinal part is seed which contain volatile oil composed of monoterpene hydrocarbons, oxygenated mono- and sesqui-terpenes, aldehydes, ketones and esters.

The present study aims to ensure the quality evaluation of the Siddha herbal formulation, Injiirasayanam with respect to its botanical, physico-chemical and HPTLC fingerprinting aspects.

## 2. Materials and methods

### 2.1. Standard Operating Procedure of the formulation

The rhizome of *Z. officinale* was collected, authenticated by Pharmacognosy Department and then scrapped to peel off the skin, cut into small pieces, 100 gms of pieces dried in shade for 30minutes and fried in Ney of required quantity. Then it was ground in Kalvam to get the fibre free confection of Inji.50 gms of Cheerakam – fruit of *C. cyminum* is cleaned, fried and then pound into the fine powder of particle size 80-100 mesh, then it is added to the above said Injiconfection and ground well. 150gms of Charkkarai was cleaned and scrapped, mixed with the above said confection and minced well for 2 hours in order to get the fibre free formulation namely Injiirasayanam (Figure 1).

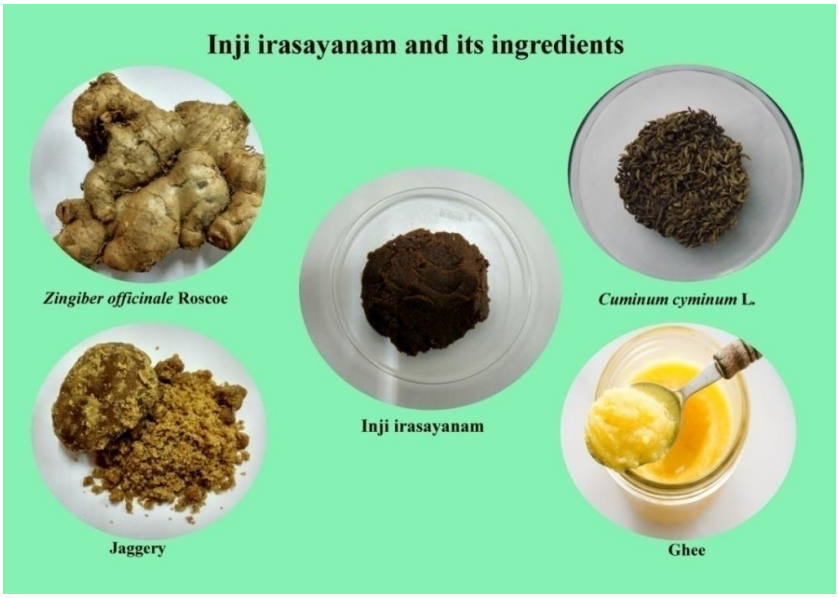


**Fig 1:** Injiirasayanam and its ingredients

### 2.2. Microscopical studies

Freehand sections of *Z. officinale* and *C. cyminum* were used for the anatomical studies. These sections were stained with safranin, mounted in glycerin and observed under the 40X objective of the light microscope.

The powdered form of Injiirasayanam was mounted in glycerin at room temperature for 24 h and observed under 20X and 40X objective of bright field microscope for powder characteristics.

### 2.3. Extraction

Alcoholic extract of Injiirasayanam was taken by reflexing the material at temperature of 60 °C for 10 minutes.

### 2.4. Physico-chemical studies

Physico-chemical constants like total ash value, water soluble extractive value, alcohol soluble extractive value and loss on drying at 105 $^0$C were determined as per standard protocol [4].

### 2.5. HPTLC fingerprinting

The ethanolic extract of Injiirasayanam was subjected to HPTLC analysis. The instrument employed was CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4. Volume of sample applied were Track 1- 5 µl; Track 2 – 10 µl. The plate was developed using solvent systems (toluene: ethylacetate: formic acid-5:2:0.1v/v) in a twin trough chamber. The plate was developed up to 8 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. at$\lambda_{max}$ 254 nm using deuterium light source, the slit dimensions were 8.00 mm × 0.40 mm. The chromatograms were recorded. After scanning, the plate was observed under 254and 366nm and then dipped in vanillin-sulfuric acid reagent and dried at 105 °C on a hot plate till the color of the spots appears. The $R_f$ values and fingerprint data were recorded by win CATS software.

## 3. Results and Discussion

### 3.1. Anatomical characters of *Z. officinale* and *C. cyminum*

As a part of standardization study, the examination of *Z. offinale* and *C. cyminum* were studied. Microscopical evaluation is a technique of qualitative evaluation based on the study of anatomical and other characters of cell inclusions, which can serve as diagnostic parameters. The following are the cellular characteristics observed.

#### 3.1.1. *Z. officinale* (rhizome)

- The cross section of the rhizome showed a zone of cork tissue which is differentiated on the arrangement of cells. The outer zone of cortical cells in the cork is suberized without division and hence is irregularly arranged. The inner zone contains cortical cells which is arranged in a radial row and produced by tangential division.
- Inner to the cork cells is the cortex composed of cortical cells. The cortical cells contain plenty of simple, ovoid starch grains which can be stained with iodine. Inner to the cork is a broad cortex.
- The outer cortex is composed of mainly flattened parenchyma, while the inner zone is composed of mainly normal parenchyma. The cortex also holds suberized oil cells which possess a yellow-brown oleoresin.
- The inner cortex contains generally three layers of closed, collateral vascular bundles which contain phloem. The larger vascular bundles are protected in a sheath of non-lignified fibres. Inner to the cortex lies a single layer of endodermis which is devoid of starch.
- Going inwards further from the endodermis lies the outermost layer of the stele which is characterized by a

single-layered pericycle. The vascular bundles of the stele resemble that of the cortex with the exception of a ring of small scattered bundles within the pericycle. The stele is mainly composed of parenchyma containing starch and oil cells similar to the cortical parenchyma.

#### 3.1.2. *C. cyminum* (seed)

- The transverse section of the *C. cyminum* seed consists of five primary ridges. Epicarp consists of single layer of elongated cells. The middle layer was mesocarp made up of thin walled parenchymatous cells. Small dark reddish brown cells were present in the mesocarp region, known as vittae.
- Two types of the sclereids were found in the mesocarp region, one was single layer, thick walled, longitudinally elongated cells and other was small groups of cells, composed of considerably elongated cells; the sesclereids have thickened walls and few pits.
- Innermost dark colored endocarp layer and inner to endocarp testa were present; it consists of single layered compact cells. Endosperm contains microspheroidal calcium oxalate crystals as observed by powder microscopical studies.

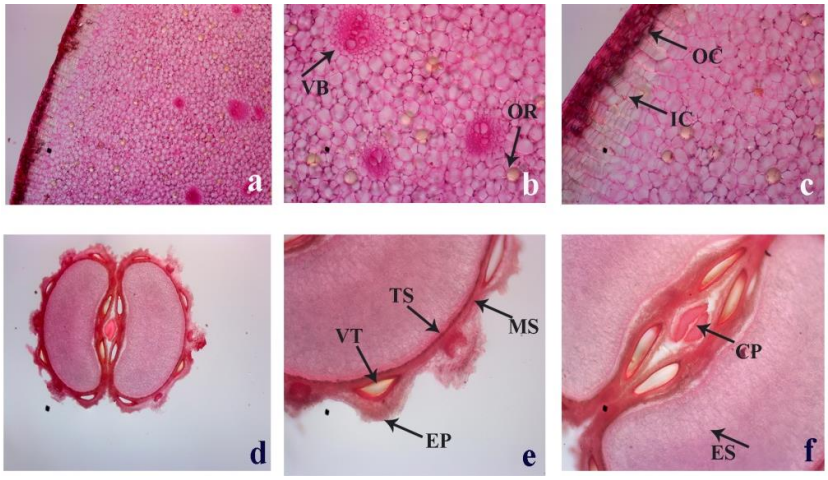


**Fig 2:** Anatomical characteristics of *Z. officinale* (a-c) and *C. cyminum* (d-f). VB-vascular bundle, OR-oleoresin, OC-outer cork cells, IC-inner cork cells, VT-vittae, TS-testa, MS-mesocarp, EP- epicarp, ES-endosperm, CP-carpophore.

### 3.2. Powder microscopical characters of Injiirasayanam

In the present study, various powder characteristics of *Z. officinale* and *C. cyminum* were observed (Fig. 3). Cork cells (outer thick walled cells and inner thin walled cells), starch grains, vessels with spiral thickenings come from *Z. officinale* rhizome. Sclereids, endosperm cells, mesocarp cells, endosperm with calcium oxalate crystals are from *C. cyminum.*

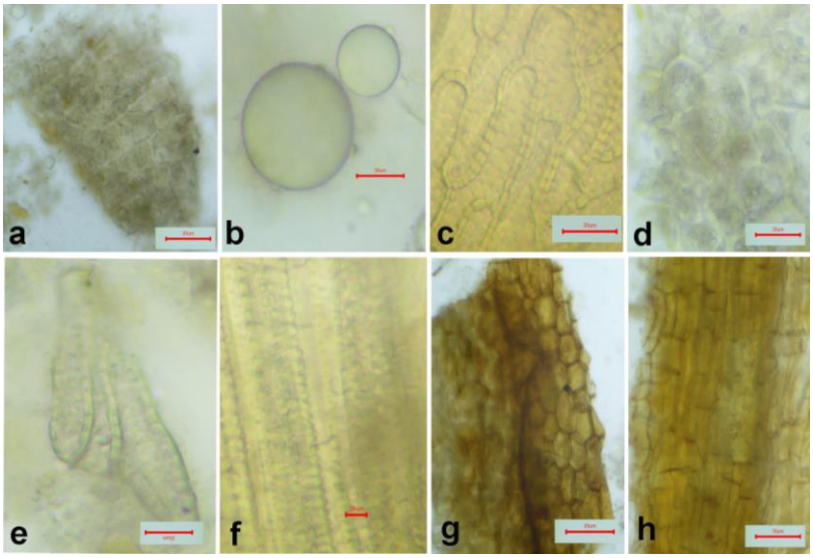


**Fig 3:** Powder characteristics of Injiirasayanam. **a:** endosperm cells, **b:** starch grains, **c:** sclerenchymatous layer of the mesocarp, **d:** endosperm cells containing microsperoidaal crystals of calcium oxalate, **e:** sclereids, **f:** vessels with spiral thickening, **g:** outer cork cells, **h:** inner cork cells

### 3.3. Physico-chemical characters of Injiirasayanam

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration. One of the important parameters in the evaluation of crude drugsis the ash value - the total ash content and the acid insoluble ash value. The total ash is particularly important in the evaluation of purity of drugs, i.e., the presence or absence of foreign inorganic matter such as metallic impurities and/or silica [5]. Total ash value and acid insoluble ash value was observed to be 3.65 and 0.23% respectively. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also

helpful in estimation of specific constituents soluble in a particular solvent. Extractive values of the plant with different solvents give a preliminary idea of the percentage of the compounds extracted [6]. Water and alcohol yielded 58.05 and 18.84% extractive (Table 1), among this water is more efficient to extract most of the phytoconstituents from the formulation. The aqueous extraction is the most common and effective method for the preparations of medicinal plant based drugs.

**Table 1:** The physico-chemical constants of Injiirasayanam

| Sl No. | Physico-chemical constants | Observations (%) |
|---|---|---|
| 1. | Water soluble extractive | 58.057 |
| 2. | Alcohol soluble extractive | 18.84 |
| 3. | Loss on drying at 105 °C | 14.75 |
| 4. | Total ash value | 3.65 |
| 5. | Acid insoluble ash | 0.23 |

### 3.4. HPTLC fingerprinting

The results indicated that the formulation contain an appreciable amount of bioactive compounds. The developed HPTLC plate of extract of Injiirasayanam at 254, 366 and after derivatization at 575 nm were represented Fig. 4a-c.

The HPTLC profile of ethanolic extract of inji irasayanam at short wavelength (254 nm) recorded is shown in Fig. 4a&4d; and the $R_f$ values and peak area percentages of the observed bands are as represented by Table 2. At 254 nm, 8 bands were obtained, out of which 3 dominant bands with brown color were observed with $R_f$ values0.15, 0.25 and 0.87(Table 3)in which the highest concentration of the phyto-constituent was found to be 64.48 % and its corresponding $R_f$ value was found to be 0.87.

At 366 nm, bands with $R_f$ values 0.13, 0.19, 0.31, 0.45, 0.91 were obtained with respect to their relative abundance and peak area percentages. All the bands observed in this wavelength were appeared to be purple colored (Fig. 4b).

After derivatization at 575 nm, yellow and purple colored bands were obtained (Fig. 4c). Their corresponding $R_f$ values were 0.11, 0.16, 0.28, 0.42, 0.51, 0.64, 0.72 and0.84 respectively. The densitometric HPTLC finger print profiles (Fig. 4e& 5a-d) may be used as marker for quality evaluation and standardization of the drug. Thus, HPTL C finger print profile along with their $R_f$ values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant [7]. The compounds corresponding to the spots observed may be responsible to render various bioactivities possessed by the herbal formulation.

**Table 2:** $R_f$ values and peak area percentages of the observed bands at 254 nm

Track 2, ID: Inji rasayanam alcohol

| Peak | Start Position | Start Height | Max Position | Max Height | Max % | End Position | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.01 Rf | 194.9 AU | 0.00 Rf | 349.7 AU | 25.01 % | 0.02 Rf | 0.1 AU | 4538.7 AU | 10.15 % |
| 2 | 0.05 Rf | 0.7 AU | 0.09 Rf | 223.2 AU | 15.96 % | 0.12 Rf | 0.6 AU | 3633.9 AU | 8.13 % |
| 3 | 0.12 Rf | 0.1 AU | 0.15 Rf | 33.7 AU | 2.41 % | 0.19 Rf | 5.8 AU | 717.3 AU | 1.60 % |
| 4 | 0.19 Rf | 6.1 AU | 0.25 Rf | 191.3 AU | 13.68 % | 0.29 Rf | 4.2 AU | 4708.3 AU | 10.53 % |
| 5 | 0.41 Rf | 1.8 AU | 0.46 Rf | 23.1 AU | 1.65 % | 0.48 Rf | 10.1 AU | 654.2 AU | 1.46 % |
| 6 | 0.49 Rf | 9.8 AU | 0.50 Rf | 13.0 AU | 0.93 % | 0.55 Rf | 0.0 AU | 264.8 AU | 0.59 % |
| 7 | 0.55 Rf | 1.9 AU | 0.59 Rf | 26.1 AU | 1.87 % | 0.62 Rf | 18.9 AU | 638.3 AU | 1.43 % |
| 8 | 0.66 Rf | 17.8 AU | 0.69 Rf | 33.1 AU | 2.36 % | 0.70 Rf | 31.7 AU | 722.2 AU | 1.62 % |
| 9 | 0.75 Rf | 36.1 AU | 0.87 Rf | 505.0 AU | 36.12 % | 1.00 Rf | 2.7 AU | 28829.2 AU | 64.48 % |

**Table 3:** $R_f$ values and colour of bands obtained at different wavelengths

| Wavelength (nm) | $R_f$ value | Colour of the band |
|---|---|---|
| 254 | 0.15 | Light brown |
| | 0.25 | Light brown |
| | 0.87 | Dark brown |
| 366 | 0.13 | Dark purple |
| | 0.19 | Light blue |
| | 0.31 | Dark blue |
| | 0.45 | Light blue |
| | 0.91 | Light blue |
| 575 | 0.11 | Yellow |
| | 0.16 | purple |
| | 0.28 | light purple |
| | 0.42 | purple |
| | 0.51 | purple |
| | 0.64 | purple |
| | 0.72 | yellow |
| | 0. 84 | purple |

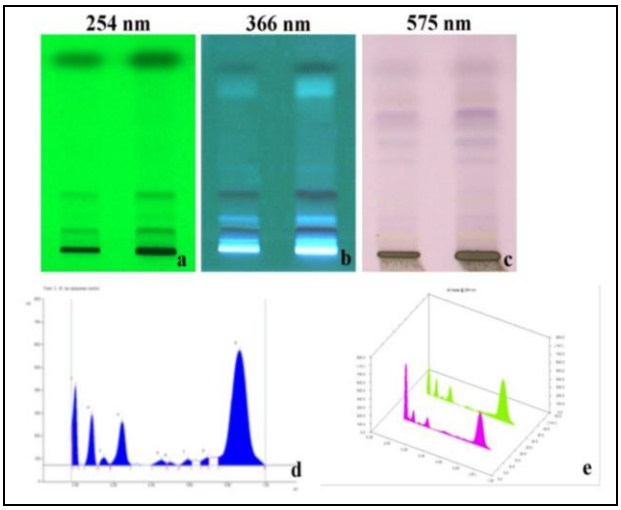


**Fig 4:** HPTLC profile of ethanolic extract of Injiirasayanam. Developed plate **a:** at 254 nm, **b:** 366 nm, **c:** 575 nm, **d:** Chromatogram and **e:** densitogramat 254 nm.

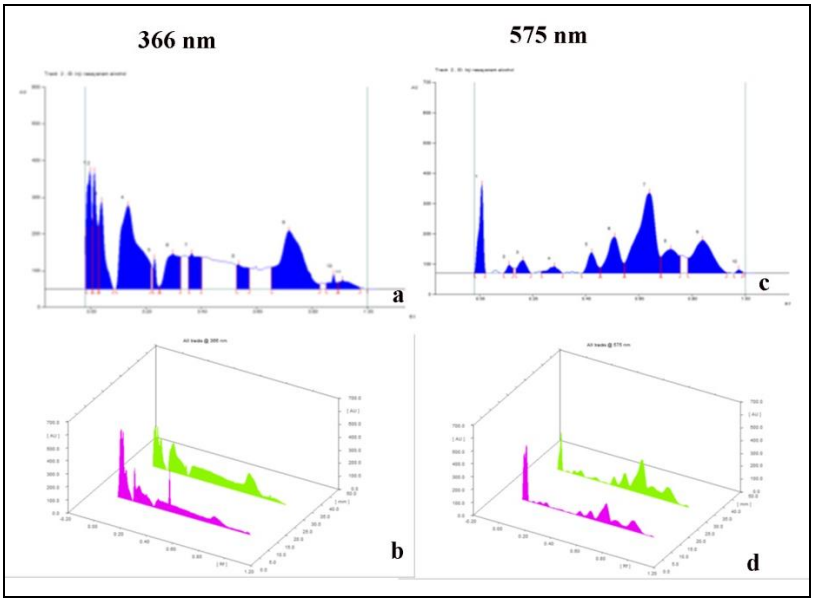


**Fig 5:** HPTLC profile of ethanolic extract of Injiirasayanam. **a:** Chromatogram and **b:** densitogramat 366 nm, **c:** Chromatogram and **d:** densitogramat 575 nm.

## 4. Conclusions

The present study on the pharmacognostic standardization and evaluation of the injiirasayanam which might be useful to contribute information with regard to its identification parameters, which are very essential and significant for the acceptability of herbal drugs in the current drug market to ensure the quality and safety of herbal drugs. The HPTLC profiles can be used for the identification and evaluation of the quality of the herbal formulation.

## 5. Acknowledgement

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