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Research Article

STANDARDIZATION OF A SIDDHA FORMULATION – SEERAGA THAILAM

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ABSTRACT

Thailams/Tailas are important group of formulations used by Siddha and Ayurvedic physicians to treat various types of diseases. Seeraga Thailam formulation which is used for the treatment of various types of head ache, fatique, insomnia and vomiting disorders was prepared as per the formula given in Siddha Formulary of India, Part II, First Edition, by procuring all the drugs from the local market. It is the favourite oil for massage as its chemical structure gives it a unique ability to penetrate the skin easily, nourishing and detoxifying even the deepest tissue layers. The formulation was studied and described along with physico-chemical parameters in authentication for quality control. The purchased cumin fruit was investigated for it authentication, morphology and microscopical characters. All the Pharmacognostical characters were found to correlate with the cumin fruit available in the standard Text books and Journals. Seeraga Thailam was subjected to various physic-chemical evaluations as described in Standard Pharmacopoeias such as Ayurvedic Pharmacopeia, Indian Pharmacopoeia, Siddha Pharmacoepia etc. Seeraga Thailam was further validated by using HPTLC Analysis. The major component of the cumin fruit is cuminicaldehyde and hence this compound was selected as a marker compound for Seeraga Thailam HPTLC Analysis and a finger print profile was prepared. Both prepared Seeraga Thailam and marketed Seeraga Thailam formulations showed similar results except mild variation in the Physico chemical parameters.

Keywords: Seeraga Thailam, Siddha Formulary of India, HPTLC Analysis.

INTRODUCTION

Tailas/Thailams are medicated oils forming a group of drugs in Siddha/Ayurvedic system of medicine. The principle is to extract the therapeutic compounds into oil. The method of preparation requires heating of oil with prescribed kashayas (decoction) and kalkas (powdered drug) according to formula. The thailams have the color; odor and taste of the drugs used, and also have the consistency of the oil. Generally they are used for abhyanga (external application) some of them are also used internally with anupanas. Thailams are medicated oils. They are similar to ghirta, but are prepared with oils instead of ghee. Usually in the preparation of thailam, sesame oil is used excepting in a few preparations where coconut oil (or) neem oil are used. Rarely mustard oil, chaulmoogra oil etc., are also used. The preparation of thailam involves different oils in various medicines as the base material to extract the active chemicals from new drugs. Majority of Siddha/Ayurvedic formulations use whole plants either alone or in combinations. The efficacy of the Siddha/Ayurvedic formulation may vary with the use of the adulterants in the formulations. It is therefore important to establish characteristics of the raw material and finished Siddha /Ayurvedic products with the help of physical and chemical methods. The world Health Organization (WHO) has emphasized the need to ensure the quality of the medicinal plants products by using modern controlled techniques and applying suitable standards. WHO in a number of resolutions has emphasized on the need to ensure the quality of herbs and herbal formulations by using modern techniques. In India as well as in other countries, several Pharmacopoeias have been published which provides monographs stating quality parameters and standards of many herbs and herbal products[1].

In order to rationalize the use of natural product extracts in therapy, a need-based and novel concept of biomarkers is being coined and defined. The bio-makers in combination with other chemical entities via poorly understood mechanisms of synergy or antagonism are supposedly responsible for the efficacy of the standardized extracts for any particular therapeutic area or disease. The bioactive extract, being the composite mixture in terms of classes and groups of organic compounds in conjunction with many extraneous materials (both organic and inorganic), could be better understood in terms of biomarker concept. Defining of the biomarker has to be very specific and a lot of insight has to go into it before declaring any distinct molecule as a biomarker. Moreover, a good number of biomarker is desirably required to achieve the elusive goal for the rational use of any given standardized extract[2].

Fingerprint analysis by HPTLC or HPLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can also be used to detect adulterations in raw materials. From the constituent profile, a number of marker compounds can be chosen which might be used to further describe the quality of the herb or the herbal preparation. High performance thin layer chromatography can also be employed for quantitative determination of such marker compounds[3].

Some of the plants are known to contain characterizing compounds that are specific to the species or family. These characterizing compounds are referred to as the chemical marker compounds which are biologically or therapeutically active principles. The plant material can be standardized with these markers and quantified-in-the-plant. In case of processed plant materials the quantification is done with the changes in the quantities of these marker compounds. Normally these chemical marker compounds are available amongst alkaloidal and some types of glycosidic crude drugs. Majority of chemical composition of higher plants in general is common to all plants and we need to direct our efforts to develop assay procedures for such type of components.

Commercially, our country has lagged far behind in the international market for traditional drugs. The use of genuine and authentic plant material is essential for production of quality drugs in these systems of medicine. In fact credibility of these systems depends upon the availability and authenticity of crude drugs[4]. One of the best methods of standardizing herbs and herbal formulations based on modern scientific tools is chromatography. It not only helps in establishing the correct botanical identity but also helps in regulating the sanctity of the herb[5]. Previous work on "Ciraka tailam" has not reported HPTLC Analysis and did not produce finger print profile for proper authentication of the formulation. [6]

Keeping in view the importance of standardization of herbal drugs, the present study was carried out with the following objectives

- To develop a standardization protocol for the selected Seeragathailam formulation from Siddha Formulary of India.
- To carry out Pharmacognostical and Physicochemical evaluation of in-house prepared formulation to arrive at quality control parameter.
- To identify marker compound present in the formulation.
- To develop a chromatographic method for the selected formulation.

MATERIALS AND METHODS

The dried cumin fruits (Seeragam) and Gingelly oil were purchased from the local grocery shop in Villuppuram, Tamil Nadu and the drugs were authenticated by the Professor of Pharmacognosy, Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu. The standard Seeraga Thailam formulation was purchased from the local Medical Stores which was manufactured by IMPCOPS Ltd., Chennai, Tamil nadu. The standard drug pure Cuminicaldehyde compound was purchased from Sigma (Aldrich), Bangaluru, Karnataka and Assigned purity: 98%. The vanillin reagent used for visualization was from Merck (Germany), and the solvents (toluene and ethyl acetate) were from Sigma (Aldrich). All chemicals used were of analytical grade. The vanillin sulphuric acid agent was prepared accordance with standard Text Book.

PROFILE OF INGREDIENTS [7]

1.Seeragam (*Cuminum cyminum;* Fam: Umbelliferae)

Seeragam consists of ripe fruits of *Cuminum cyminum*, Linn. (Fam.Umbelliferae), a glabrous, annual herb, 30-90 cm hight, flowers very small, white, about 38 mm long stalk in compound umbels, mostly cultivated in plains, plants pulled out, dried thrashed for collecting mature fruits. In Indian recipes, cumin is frequently confused with caraway, which it resembles in appearance though not in taste, cumin being far

more powerful. This is due to a misunderstanding of the Indian word "jeera". The term usually means cumin, but can occasionally mean caraway, so in doubtful cases, cumin is generally to be understood. The use of the terms 'black cumin' for nigella, and 'sweet cumin' for aniseed or fennel, further confounds this confusion. As a general rule interpret jeera or zeera (jira, zira) as cumin and kalonji as nigella. When the seeds themselves are in doubt, cumin is easily distinguished from the other Umbelliferae by its flavour, and its shape and colour is quite different from nigella.

Uses of Cumin

Traditional uses of cumin include to reduce inflammation, increase urination, prevent gas, and suppress muscle spasms. It has also been used as an aid for indigestion, jaundice, diarrhea, and flatulence. Cumin powder has been used as a poultice and suppository, and has been taken orally.The oil, which is derived by steam distillation, is used to flavor alcoholic beverages, desserts, and condiments. It is also used as a fragrant component of creams, lotions, and perfumes.

2. Gingelly oil (Sesame oil)

The rich, almost odourless oil expressed from the tiny seeds is very stable and contains an antioxidant system comprising sesamol and sesamolinol formed from sesamolin, which substantially reduce its oxidation rate.It is also highly nutritious, rich in vitamins A, B and E as well as the minerals iron, calcium, magnesium, copper, silicic acid and phosphorus. It contains linoleic acid and alpha linoleic acid as well as lecithin, and this may go some way to explaining its benefit to the brain and nervous system. Like olive oil, sesame oil is considered good for lowering harmful cholesterol levels.

Uses of Gingelly oil (Sesame oil)

Sesame oil is immensely popular in India where its use in oil massage (abhyanga) is part of everyday life and an important aspect of Ayurveda. It is the favourite oil for massage as its chemical structure gives it a unique ability to penetrate the skin easily, nourishing and detoxifying even the deepest tissue layers. In fact it is said to benefit all the seven tissues (Dhatus). It is the best oil for balancing *Vata* but can also be used sparingly for *Pitta* and *Kapha*.

Used regularly, sesame oil is wonderful for reducing stress and tension, nourishing the nervous system and preventing nervous disorders, relieving fatigue and insomnia, and promoting strength and vitality. Those patients who use sesame oil daily have reported feeling stronger, more resilient to stress, with increased energy and better resistance to infection. Its relaxing properties ease pain and muscle spasm, such as sciatica, dysmenorrhoea, colic, backache and joint pain.

PREPARATION OF STANDARD SEERAGA THAILAM FORMULATION[8] Ingredients:

1. Seeragam : 35 G
2. Gingelly oil : 1.400 L

Method of Preparation

The ingredients were mixed and boiled till Seeragam was fried and cooled. The resulting Thailam was filtered through muslin cloth and stored in a clean container at room temperature for further analysis.

Organoleptic Characters

The organoleptic characters of Seeraga Thailam; both prepared and purchased from the market were presented in the Table 1. Organoleptic tests are very important because palatability of a drug depends upon these characters.

Physico chemical parameters of S e e r a g a Thailam (Table 2)

The following Physico chemical parameter studies and HPTLC analysis were carried out for both Seeraga Thailam prepared in the laboratory as well as for the purchased one from the market. All the investigations of physico chemical parameters were carried out as per the methods described in Indian Pharmacopoeia (2014), 7th Edition published by Govt. of India.

Specific gravity

Specific gravity is the ratio of the density of a substance compared to the density (mass of the same unit volume) of a reference substance. Apparent specific gravity is the ratio of the weight of a volume of the substance to the weight of an equal volume of the reference substance. The reference substance is nearly always water for liquids or air for gases. Temperature and pressure must be specified for both the sample and the reference.

Weight per mL

The wt/ml of a liquid is the weight in gram of 1 ml of a liquid when in air at 25°C unless otherwise specified. The weight per milliliter was determined by dividing the weight in air, in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature

Acid value
The acid value is defined as the number of milligram of potassium hydroxide required to neutralize the free acid present in 1 g of oil or fat.

Saponification value
The saponification value is the number which expresses in milligrams the amount of potassium hydroxide necessary to neutralize the free acid and to saponify the ester present in 1 g of fat or oil.

Unsaponification value
It consists of substance present in oils and fats which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance under examination.

Iodine value
The iodine value of a substance is the weight of halogens expressed as iodine absorbed by 100 parts by weight of the substance. The quantity of substance used in the determination should be such that at least 70% of the iodine added, as provided in the recommended procedure, is not absorbed.

Refractive Index
The refractive index of a substance is the ratio of the speed of light in a vacuum to the speed of light in the substance. For practical measurements, including this method, the scales of standard instruments indicate refractive indices with respect to air rather than vacuum.

Viscosity
Viscosity of a liquid is constant at a given temperature and is an index of its composition. Hence, it can be used as a means of standardizing liquid drugs**.**

HPTLC ANALYSIS[9]
Instrumentation & conditions
Preparation of the samples for Chromatography analysis
Both purchased and marketed Thailams were dissolved in chloroform and filtered through Whatmann Filter paper and the volume was reduced to 5ml. 1ml of **Cuminicaldehyde** was dissolved in 10ml alcohol (95%) and used as a marker compound.

Procedure
The samples were spotted in the form of bands of width 3mm with a Camag micro litre syringe on precoated silica gel aluminium plate 60 F254 (10 cm × 10 cm with 0.2 mm thickness; E. Merck, Darmstad, Germany) using a Camag Linomat V (CAMAG, Muttenz, Switzerland). A constant application rate of 150 nL/s was employed and space between two bands was 4 mm. The slit dimension was kept at 4 mm × 0.1 mm, and 20 mm/s scanning speed was employed. These parameters were kept constant throughout the analysis of samples. The mobile phase consisted of **toluene and ethyl acetate in a ratio of 9: 3 *v/v*.** Plates were developed in ascending order with a CAMAG twin trough glass tank which was pre-saturated with the mobile phase for 15 min; the length of each run was 8 cm. The TLC runs were performed under laboratory conditions of (Temp: 25 ± 2°C and % RH: 60 ± 5). The plates were then dried in air. Densitometric analysis was performed at 513 nm with a Camag TLC scanner III operated by Win CATS software (Version 1.2.0). The source of radiation utilized was deuterium and tungsten lamp. The composition of the mobile phase for TLC was optimized using different solvents of varying polarity and good resolution was achieved using toluene: ethyl acetate (9: 3 *v/v)* as mobile phase. The *R*f value for cuminicaldehyde was found to be 0.62 ± 0.02. The scanning wavelength selected was 513 nm, the absorption maxima of the cuminicaldehyde spot. The Finger print profile of the samples developed in the HPTLC plate was photographed after spraying with vanillin - sulphuric acid agent. [Fig: 1]

RESULTS OF HPTLC INVESTIGATION
Selection and optimization of mobile phase
Initially toluene: ethyl acetate in varying ratios was investigated. The mobile phase Toluene: ethyl acetate (9:3.5: *v/v*) gave good resolution with *R*f value of 0.62 ± 0.02 for cuminicaldehyde but typical peak nature was missing. The volume ratios of ethyl acetate of over said system was varied to determine the effect on *R*f and on the response to cuminicaldehyde. Finally, the mobile phase consisting of Toluene: ethyl acetate (9: 3 *v/v*) gave a sharp and well-defined peak at *R*f value of 0.62 ± 0.02 (Fig 05). Well-defined spots were obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.
Cuminicaldehyde was found to be major constituent of Cumin volatile oil. Hence, cuminicaldehyde was selected as a marker compound for this formulation.
Cuminicaldehyde was shown in all the formulations (3, 6 and 7). Spot 3 which is cumin extract showed 5 distinctive spots while lab prepared formulation as well as marketed formulation showed only three distinctive spots with same Rf values. This may be due to the

boiling of oil with cumin fruits and thereby missing of certain active constituents which are present in very low quantities.

SUMMARY AND CONCLUSION

The field of the herbal drugs and formulations is very vast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal drug formulation it is must to have all the related knowledge of that particular drug including all its organoleptic characters to phytoconstituents to pharmacological action to its standardization in respect to various parameters via various techniques[10]. Monographs as compiled in the standard books like Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India, Wealth of India and Ayurvedic formulary, Siddha Formulary etc., provide all the details for the various tests to be performed in order to determine the conformity of the crude or formulated herbal drug with the standards lay. It is also important to standardize, not only the main drug constituent but also the other excipients and additives incorporated.

The Siddha formulation Seeraga Thailam was studied and described along with physico-chemical parameters in authentication for quality control. The purchased cumin fruit was investigated for it authentication, Morphology and Microscopical characters. All the Pharmacognostical characters were found to correlate with the cumin fruit available in the standard Text books and Journals. Seeraga Thailam was prepared as per the direction given in Siddha Formulary of India; a Govt. of India Publications. Seeraga Thailam exhibited a set of diagnostic characters, which will help to identify the drug in the Siddha formulations. Seeraga Thailam was subjected to various physic-chemical evaluation as described in Standard Pharmacopoeias such as Ayurvedic Pharmacopeia, Indian Pharmacopoeia, Siddha Pharmacoepia etc. Seeraga Thailam was further validated by using HPTLC Analysis.

Fingerprint analysis by HPTLC or HPLC is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. In combination with microscopic investigations, the fingerprint provides the means for a convenient identity check. It can also be used to detect adulterations in raw materials. From the constituent profile, a number of marker compounds can be chosen which might be used to further describe the quality of the herb or the herbal preparation. High performance thin layer chromatography can also be employed for quantitative determination of such marker compounds. The major component of the cumin fruit is cuminicaldehyde and hence this compound was selected as a marker compound for Seeraga Thailam HPTLC Analysis. Both prepared Seeraga Thailam and marketed Seeraga Thailam formulations showed similar results except mild variation in the Physico chemical parameters as shown in Table:2.

The various analytical data generated here may be considered as the standard parameters for the formulation. The finger print profile may be kept as a standard for evaluating future samples of Seeraga Thailam.

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RESULTS OF ORGANOLEPTIC & PHYSICO-CHEMICAL PARAMETERS OF SEERAGA THAILAM [9]

Table 1: Organoleptic characters of Seeraga Thailam

Serial No	Parameters	Prepared Seeraga Thailam	Marketed Seeraga Thailam
1	Colour	Yellow	Dark Yellow
2	Odour	Pleasant	Pleasant
3	Appearance	Translucent	Translucent
4	Taste	Bitter	Intense bitter
5	Touch	Oily	Oily

Table 2: Physico-chemical parameters of Seeraga Thailam

Serial No	Parameters	Prepared Seeraga Thailam	Marketed Seeraga Thailam
1	Specific gravity	0.9165	0.9195
2	Wt/ml	0.9125	0.9225
3	Refractive index	1.4644	1.4544
4	Acid value	2.6	3.1
5	Saponification Value	156.66	157.66
6	Unsaponification Value	1.50	1.60
7	Iodine Value	101	106
8	Viscosity	35.33 mm2/sec	35.88 mm2/sec

Finger Print Profile of Seeraga Thailam (HPTLC Analysis)

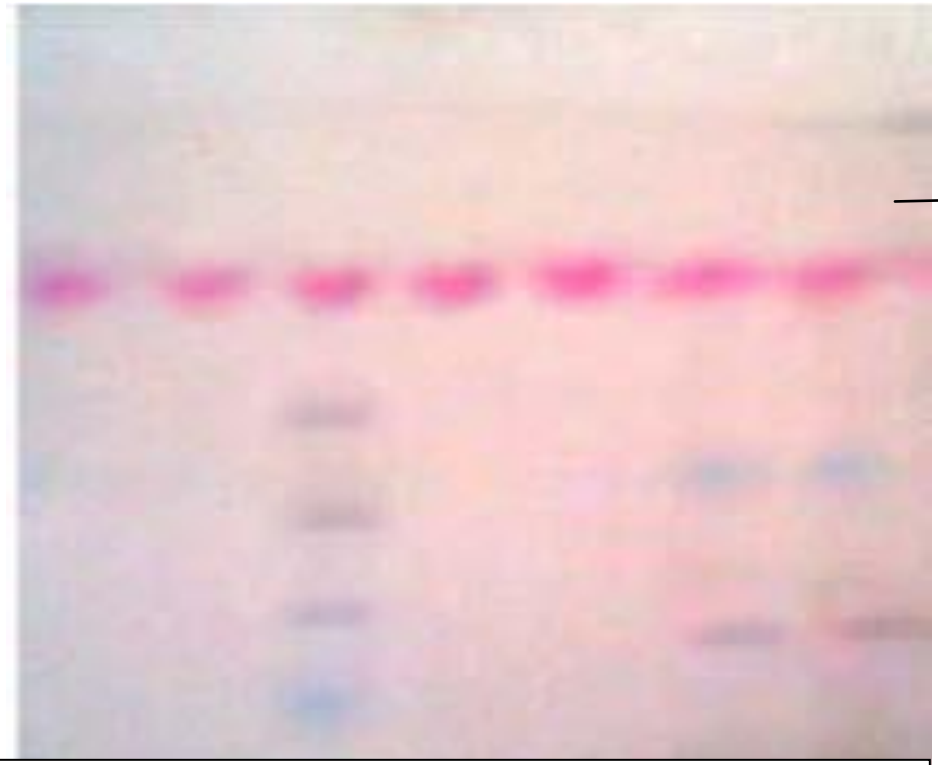

Fig. 01: Developed HPTLC Plate at Vanillin sulphuric acid agent, 1, 2, are standard cuminaldehyde (1 µml/spot), 3 is Cumin extract (10 µml/spot), 4, 5 standard cuminaldehyde (2 µml/spot), 6 is prepared formulation extract (10µg/spot), 7 is marketed formulation extract (10 µml/spot)

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