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Physio Chemical Standardization of Vasavennai – Siddha Medicine

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ABSTRACT

Background: Siddha system of medicine having unique type of treatment called Varmam therapy. Its include internal medicine and external applications also. This therapy is widely used for musculoskeletal disorders such as fractures, dislocation, sprain, strain and degenerative disorders and for neurological deficit. In above said conditions, Post manipulation phase-certain periods of immobilization, the healing process usually lead to scar formation, which can cause stiffness of the joint.

Objectives: The healing process, movement factors and further rehabilitation factors are contributing the progress of the any joint after the injury. When attempting to move after some period of inactivity can cause strain on the muscles and the tissues, making movement of joint difficult, feel stiff and results in discomfort and restriction of joint movement for a prolonged period. After Varmam manipulation in this condition, Vasavennai is one of the oil, which mentioned for lubrication and stiffness of the joint and allows free movements of joint. These not only help to improve range of motion but also help strengthen the muscles and tissues, increase flexibility, increase blood supply and reduce chances of injuries. The aim of the study is to standardize this preparation.

Results: The Physiochemical parameters of this Vasavennai, Specific gravity, acid value, Iodine value, Peroxide value, saponification value and unsaponifiable matter are 0.971g/ml, 6.46, nil, 9.735, 165.79 and 3.21 % .

KEYWORDS: Varmam Therapy, Rehabilitation, Stiffness, Vasavennai

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Page | 6

I. INTRODUCTION

In current scenario, traditional systems of medicines are gaining importance due to long historical practice in particular region. Most of the poly herbal formulations are under trial by leading pharmaceutical companies due to their clinical use traditionally for generations and its efficacy leads to global attention to Indian traditional systems^[1]

The siddha system of medicine is origin of Southern part of India; it has developed along with Tamil language. This system has unique type of treatments and medicines such as Varmam therapy, Muppu process- Vasi yogam, Astanga yogam, Kaya karpam (Elixir science) muppu kattu, kalangu, Guru kuligai etc.^[2] The varmam therapy is practiced by siddha doctors as well as traditional practioner in Tamilnadu and Kerala. This treatment methodology taught by Guru-disciple method. This varmam therapy used for various types of musculo skeletal disorders, fractures, bone settings and neurological deficit. In bone settings, lot of techniques are using for reduction of fractures and dislocation of joints. In addition, various types of medicines like tablets, oils (internal and external), pastry, fomentation for bone settings, reducing stiffness, rehabilitation of joints. Thailam or ennai are medicated oils listed under internal medicines with shelf life of one year according to Siddha literature. Tailam can be used for internal as well as external also.^[3]

Vasavennai is one of the oil mentioned in varma literature indicated for lubrication and stiffness and allows free movements of joint. These not only help to improve range of motion but also help strengthen the muscles and tissues, increase flexibility, increase blood supply and reduce chances of injuries. Literary search revealed that there are more than 40 types of vasavennai preparations are available so far. Here this preparation selected from *Siddha Maruthuvam- Sirappu*, Directorate of Indian medicine, Chennai; 2012. Page:179.^[4] No standardization work has not done on any of above types. The aim of the present study was to evaluate the organoleptic, physico-chemical, and chromatography standards of Vasavennai.

II. MATERIALS AND METHODS

Standard Operating Procedure (SOP) of Vasavennai

Table 1. Ingredients of Vasavennai

The following table represents the ingredients used to prepare this oil^[4]

Sl. No.	Name of the drug	Botanical name	Parts/products used	Quantity used
1.	Gingelly oil	Sesamum indicum	Oil	200 ml
2.	Kungliyam	Vateria indica	Resin	15 gm
3.	Aloe juice	Aloe vera	Leaf juice	200 ml

Method of preparation

Powder the kungliyam into powder, mix the aloe vera juice, oil and powder into bowl. Keep the mix in sunlight for 3 days. The duration of days depends upon the severity of heat. The above is churning with wooden churn until it become butter (semisolid) consistency. Because in Tamil language, vennai means butter. The above mix kept in sunlight again for some hours for allowing evaporation of water content if excess. The product transferred into clean airtight container. The samples taken for further analysis.

Organoleptic characters

The organoleptic characters such as colour, touch, taste and odour noted.

Physico-chemical parameters

Physico-chemical studies of the oil carried out as per standard protocol^{[5], [6]}. Physico-chemical constants such as specific gravity, acid value, peroxide value, saponification value, unsaponifiable matter and iodine value for the tailam was determined.

Determination of specific gravity:

Fill the dry pycno meter with prepared samples in such a manner to prevent entrapment of air bubbles after removing the cap of side arm. Insert the stopper, immerse in water bath at 30 °C and hold for 30 min^{[5], [6]}. Carefully wipe off any oil that has come out of the capillary opening. Remove the bottle from the bath, clean and dry it thoroughly. Remove the cap of the side and quickly weigh ensuring that the temperature does not fall below 30 °C.

Determination of acid value

Weigh 5 gm. of oil and transfer it into 250 ml conical flask. Add 50 ml of neutralized alcohol solution (25ml of alcohol and 25 ml diethyl ether) to the oil solution. Heat this mixture for 10 minutes by using the heater. Take the solution after 10 minutes and add 1 or 2 drops of phenolphthalein indicator. Titrate this against the KOH solution from the burette. The appearance of pink colour indicates the end point^{[5], [6]}.

Determination of saponification value

Weigh 1 gm. of oil and transfer into the round bottomed flask. Add 20 ml of 0.5 N alcoholic KOH solutions to the round bottomed flask. Follow the above procedure without taking oil for blank titration. Reflux both sample and blank round bottomed flasks for 1 hour. After reflux, allow both the round bottomed flasks to cool. Titrate the samples using 0.5 N HCl with phenolphthalein indicator. The disappearance of pink indicates the end point.^{[5], [6]}

Determination of unsaponifiable matter

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. [5], [6]

Determination of Peroxide value

Transfer approx. 3.0 g of the sample, accurately weighed, into a 250 ml Erlenmeyer flask with glass stopper. Add 50 ml of the glacial acetic acid: chloroform 3:2 solvent mixtures and saturated potassium iodide solution, 1 ml, freshly prepared and allow reacting for 60 seconds \pm 1 second and shaking thoroughly during this period. Then add water, 100 ml and shake. Titrate with 0.01 mol/l sodium thiosulfate solution, using 1 ml starch solution indicator. The indicator should be added towards the end of the titration but while the pale straw colour is still present. During titration shake until the blue colour disappears. Carry out a blank titration under the same conditions. [5], [6]

Development of high performance thin layer chromatographic (HPTLC) profile

HPTLC is a micro analytical separation and determination method, which has a wide application in herbal drug analysis. The unsaponifiable matter of Vasavennai, prepared by the methods of Pramod et al [7]. The unsaponifiable matter of the oil spotted in the form of bands with Camag microlitre syringe attached with Camag ATS4 instrument on a Merck Aluminium plate pre-coated with silica gel 60 F₂₅₄ of 0.2 mm thickness. Solvent systems for the studies selected by trial and error methods. The system which gave the maximum resolution were taken. The solvent system selected was Toluene: Ethyl acetate (8: 1). The plate developed in twin trough chambers pre-saturated with the selected mobile phase. The plate was developed up to 8 cm, removed from the chambers and allowed to air dry. The plate was visualised under UV 254, UV 366 in a CAMAG visualise and the images were documented. Then the plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the finger print profiles were documented. The R_f values and finger print data were recorded with win CATS software associated with the scanner. The plate derivatised using vanillin-sulphuric acid reagent, heated at 105°C until the development of coloured spots and viewed in white light and the chromatograms documented. The plates were scanned in white light (575 nm) to obtain the fingerprint profiles and R_f values [8].

III. RESULTS AND DISCUSSION

Vasavennai is an indigenous herbal formulation containing *V.indica*, *A. vera* and *Sesamun indicum* (gingelly oil) which claims to have the potential in the treatment of siddha varmam therapy.

It is being practiced by Siddha practitioners for the treatment of varmam therapy.

Organoleptic characters

Organoleptic characters such as colour, odour, appearance and touch of Vasavennai given in Table 2.

Table.2 Organoleptic evaluations of Vasavennai

Sl. No	Parameters	Results
1	Colour	Light straw yellow colour
2	Odour	Unspecified
3	Appearance	Colloidal
4	Touch	Greasy substance

Physico-chemical properties

The physico-chemical parameters of the Vasavennai and gingelly oil tabulated in below.

Table 3: Physico-chemical characters of Gingelly oil and Vasavennai

Sl. No.	Test	Gingelly oil [9]	Vasavennai
1	Specific gravity	0.943 g/mL	0.971g/mL
2	Acid value	2.918	6.46
3	Iodine value	112.1	---
4	Peroxide value	10.16	9.735
5	Saponification value	183.727	165.79
6	Unsaponifiable matter	2.59%	3.21%

The acid value defined as the number of milligram of potassium hydroxide required to neutralize the free acid present in 1 g of oil or fat. 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. The acid and iodine values depend on the extraction of acidic and unsaturated compounds into the gingelly oil from the ingredient during the process of preparation. 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. Saponification value for Gingelly oil found to be 183.727 whereas that for Vasavennai was 165.79.

Unsaponification value consists of substance present in oils and fats, which are not saponifiable by alkali and determined by extraction with an organic solvent of a solution of the saponified substance under examination. The values obtained for Gingelly oil was 2.59% and that for Vasavennai was 3.21%. The gingelly oil acts as base in the Vasavennai for the preparation of Vasavennai. These parameters may be used as standards for Vasavennai. The values obtained for gingelly oil are in agreement with the reported values ensuring the authenticity of the oil, which used for the preparation of the Vasavennai.

HPTLC fingerprinting of Vasavennai

The results showed various bioactive compounds in the chloroform extract of Vasavennai. The developed HPTLC plate at 254 nm, 366 nm and 575 nm after derivatisation using vanillin-sulphuric acid reagent represented various bands having different band intensities. The representative chromatograms are shown in Figure 2.

The R_f values and peak area percentages of the observed bands for 5µl and 10µl of chloroform extract of Vasavennai (Unsaponified matter dissolved in chloroform) at 254 nm, 366 nm and 575 nm after derivatisation are represented in Figures 3, 4, and 5 respectively.

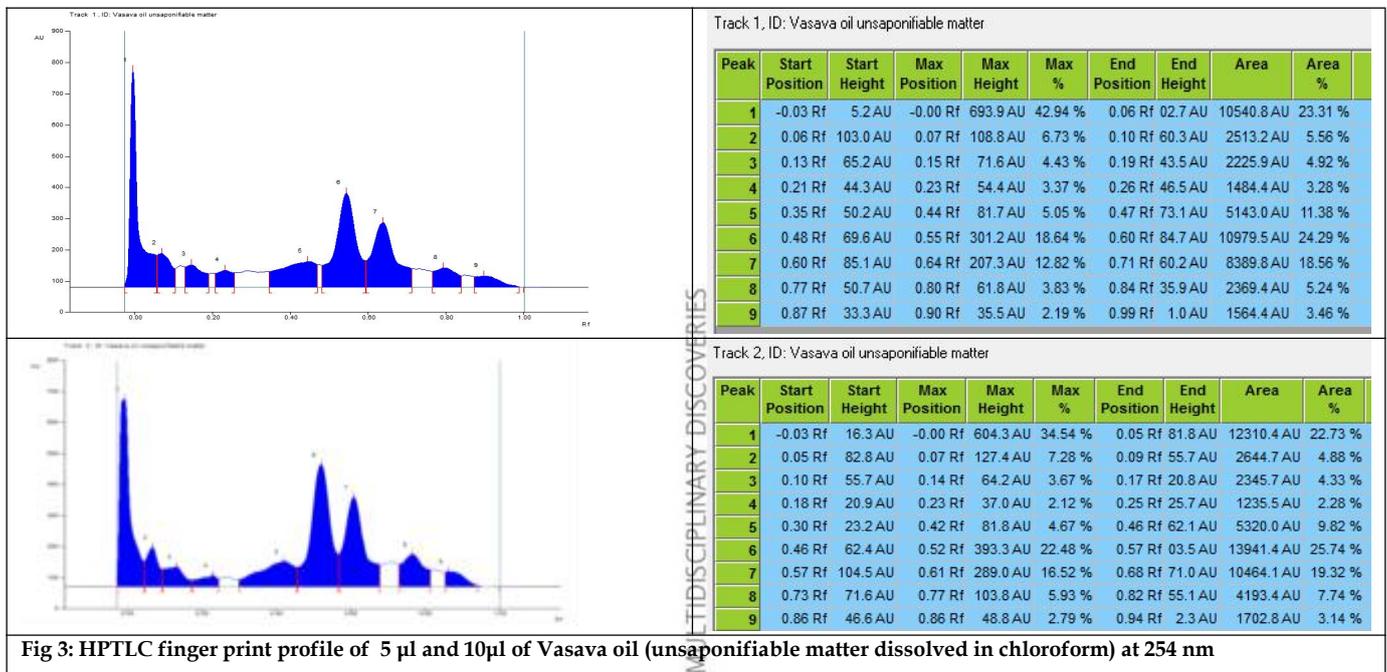


Fig 3: HPTLC finger print profile of 5 µl and 10µl of Vasava oil (unsaponifiable matter dissolved in chloroform) at 254 nm

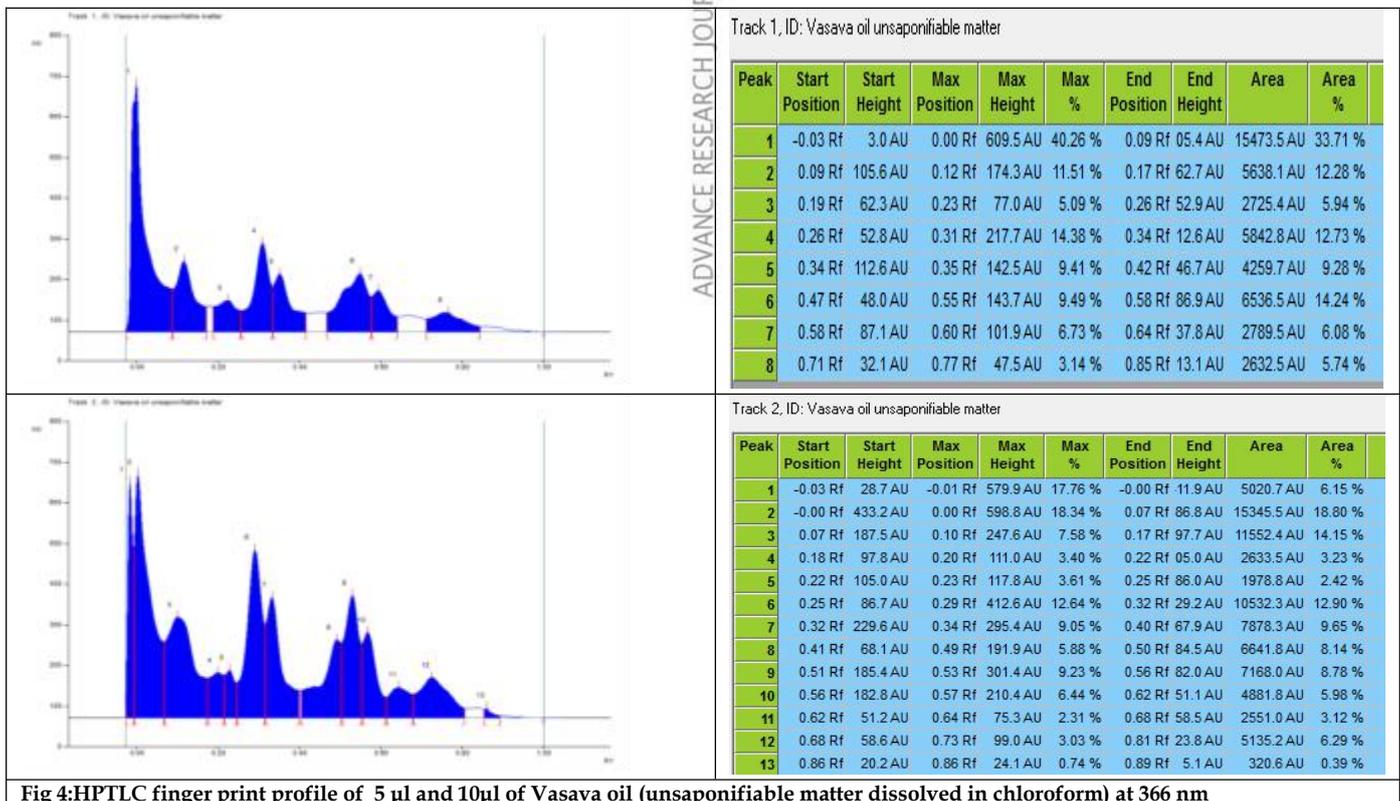


Fig 4: HPTLC finger print profile of 5 µl and 10µl of Vasava oil (unsaponifiable matter dissolved in chloroform) at 366 nm

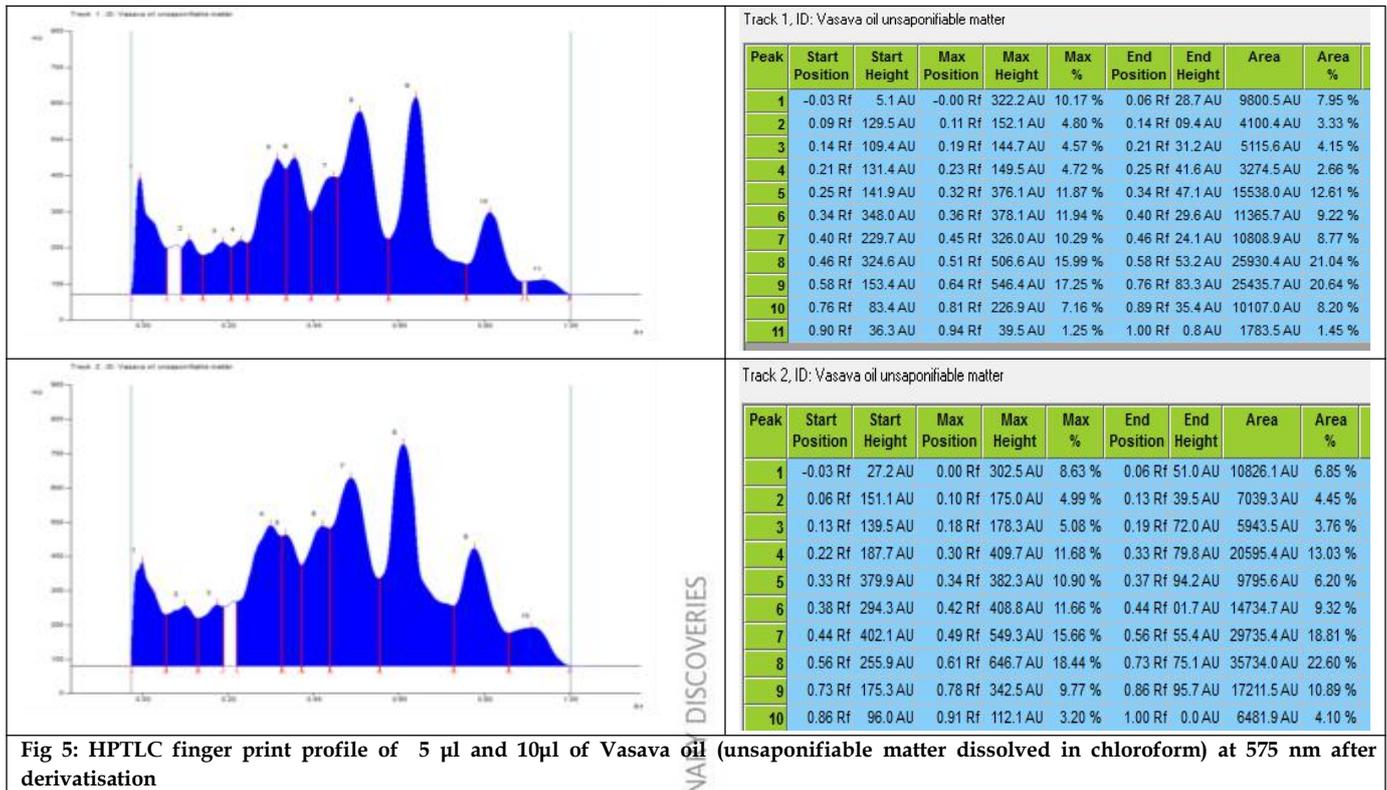


Fig 5: HPTLC finger print profile of 5 µl and 10µl of Vasava oil (unsaponifiable matter dissolved in chloroform) at 575 nm after derivatisation

The R_f values and colour of bands obtained at different wavelengths for Vasavennai and the area of spots obtained for the Vasavennai are represented in Table 4.

Table 4: R_f values, colour of bands area of bands obtained at different wavelengths for Vasavennai

Wave length (nm)	Sl.No.	R _f values	Colour	Area of spots (AU)
254 nm	1	0.07	Light green	2513.2
	2	0.15	Dark green	2225.9
	3	0.23	Light green	1484.4
	4	0.44	Light green	5143.0
	5	0.55	Dark green	10979.5
	6	0.64	Dark green	8389.8
	7	0.80	Light green	2369.4
	8	0.90	Light green	1564.4
366 nm	1	0.00	Light Blue	15345.5
	2	0.10	Blue	11552.4
	3	0.20	Blue	2633.5
	4	0.23	Blue	1978.8
	5	0.29	Blue	10532.3
	6	0.34	Blue	7878.3
	7	0.49	Blue	6641.8
	8	0.53	Blue	7168.0
	9	0.57	Blue	4881.8
	10	0.64	Blue	2551.0
	11	0.73	Blue	5135.2
	12	0.86	Blue	320.6
575 nm	1	0.11	Grey	4100.4
	2	0.19	Grey	5115.6
	3	0.23	Yellow	3274.5
	4	0.32	Purple	15538.0
	5	0.36	Purple	11365.7
	6	0.45	Pink	10808.9
	7	0.51	Grey	25930.4
	8	0.64	Blue	25435.7
	9	0.81	Pink	10107.0
	10	0.94	Grey	1783.5

HPTLC is an important quality assessment method for the evaluation of herbal drug samples and is the simplest separation technique today available to the researchers and scientists. HPTLC is a micro analytical separation and determination method, which has a wide application in herbal drug analysis. HPTLC fingerprinting profile helps very much in standardization for the proper identification of medicinal plants and compound formulations, and is suitable for their rapid decisive authentication. The HPTLC fingerprints developed in this study are able to ensure the quality of Vasavennai and provide referential information of the formulation.

IV. CONCLUSION

The above-cited physicochemical parameters can be noted as pharmacopoeial standards and will help us to determine the genuineness of Vasavennai. And also take it to check the quality of the medicine. The results of High Performance Thin Layer Chromatographic (HPTLC) studies used as a diagnostic tool to identify and to determine the quality and purity of the formulation in future studies.

V. CONFLICT OF INTEREST

No conflicts declared

VI. ACKNOWLEDGMENT

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