INTRODUCTION

Pain management in Siddha system of medicine is done by means of internal and external medicines.\(^1\) Siddha Varmam therapy, an external pressure manipulation therapy/technique, is also gaining popularity among the public in the management of pain.\(^2\) The role of external oil application in the pain management is commendable.\(^3\) Aggravated Vatham manifests as pain in the body and the mode of treatment which mitigates the aggravated vatham will certainly reduce pain.\(^4\) There are a number of internal and external formulations indicated for vatha diseases. Vatha Kesari Thailam (VKT) is one of the external medicines indicated for Rheumatalgia, Hemiplegia, Paraplegia and other Vatha diseases.\(^5\) Therapeutic effect of all the formulations depends upon the quality of raw materials used for preparation. Adulteration in raw materials will affect the therapeutic effect of any formulation.\(^6\) WHO has emphasized the development of standards for raw materials used in formulations of Siddha medicine. Govt. of India has constituted Siddha Pharmacopoeia Committee (SPC) to develop standards for Siddha formulations. Siddha Pharmacopoeia committee is engaged in the publication of Siddha Pharmacopoeia of India indicating quality parameters and standards for herbs and herbal formulations.\(^7\) The aim of this study is to evaluate the physicochemical characters of VKT as per standards mentioned in SPI.

MATERIALS AND METHODS

Vatha Kesari Thailam (VKT) was purchased from The Indian Medical Practitioners Cooperative Pharmacy and Stores Ltd (IMPCOPS), a GMP certified pharmaceutical company in Chennai.
Physicochemical parameters
The following physicochemical parameters were studied in the Department of Chemistry, Siddha Central Research Institute, Chennai. All the tests were carried out as per the methods described in the Siddha Pharmacopoeia (2014) 1st edition, Govt. of India.

1. Determination of Acid Value
The acid value is the amount of potassium hydroxide (in mg) required to neutralize the free acid in 1 g of the substance.

Method
Take accurately 10 g of the substance (1 to 5) in a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized by the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary, until the substance has completely melted, titrate with 0.1 N potassium hydroxide, shaking constantly until a pink colour which persists for fifteen seconds is obtained. Note the amount of ml required. Calculate the acid value from the following formula: —

\[
\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}
\]

Where ‘a’ is the amount (in ml.) of 0.1 N potassium hydroxide required and ‘w’ is the weight in gm of the substance taken.

2. Determination of Saponification value
The saponification value is the amount of (in mg) potassium hydroxide require to neutralize the fatty acids, resulting from the complete hydrolysis of 1 g of the oil or fat.

Method
Dissolve 35 to 40 g of potassium hydroxide in 20 ml water and add sufficient alcohol to make 1,000 ml. Allow it to stand overnight and pour off the clear liquor. Take accurately 2 g of the substance in a tared 250 ml flask, add 25 ml of the alcoholic solution of potassium hydroxide, attach a reflux condenser and boil on a water-bath for one hour, frequently rotating the contents of the flask. Allow it to cool and add 1 ml of solution of phenolphthalein and titrate the excess of alkali with 0.5 N hydrochloric acid. Note the amount (in ml) required (a) Repeat the experiment with the same quantities of the same reagents in the manner without the substance. Note the amount (in ml) required (b) Calculate the saponification value from the following formula: —
Saponification Value = $\frac{(b-a) \times 0.02805 \times 1.000}{w}$

Where ‘w’ is the weight in g of the substance taken.

3. Determination of Iodine Value

Iodine value is usually expressed as the number of parts by weight of Iodine absorbed by 100 parts by weight of an oil or fat.

Preparation of reagents

Dissolve 13.2 g of Iodine in 1 litre of Glacial acetic acid using a mortar and pestle. Filter through cotton and transfer all the iodine with Glacial acetic acid. Add 3 ml of liquid bromine and shake well.

- Dissolve 25g of Sodium thiosulphate AR in 1000ml of water in a volumetric flask
- Dissolve 4.904g of Potassium dichromate AR in 1 litre volumetric flask and make up to the mark with distilled water
- Dissolve 1g of Starch in 100 ml of distilled water. Heat till it becomes colourless.

Take 10ml of 0.1N Potassium dichromate solution add 150 ml of distilled water, 2g of potassium iodide and 6 ml of Conc. Hydrochloric acid. Stir well. Allow it to stand for 10 minutes. Titrate with Sodium thiosulphate solution using starch as indicator. End point is disappearance of blue colour.

Method

Weigh about 0.2 gm of oil/fat in an Iodine flask. Add 15 ml of Chloroform and dissolve. Add 25 ml of Iodine monobromide from a burette. Moisten the stopper with a few drops of potassium iodide solution. Keep the flask in dark for half an hour. Add 10 ml of potassium Iodide and 100 ml of distilled water. Titrate the mixture with standard 0.1M Sodium thiosulphate with starch as indicator. Add starch solution only when the solution in the flask is pale yellow in colour. The end point is disappearance of blue colour. Note the amount (in ml) required (a). Do the blank determination without the oil using exactly the same quantity of chloroform and the same burette for delivering the Hanus reagent (b). Repeat the experiment twice to get concordant values.
Calculation

Iodine value = 1.269 \times \text{Strength of thiosulphate} \times \text{Difference in titre (b-a)} \\
0.1 \times \text{Weight of oil}

4. Determination of Specific Gravity

Specific gravity – The specific gravity of a liquid is the weight of a given volume of the liquid at 25º (unless otherwise specified) compared with the weight of an equal volume of water at the same temperature, all the weighings being taken in air.

Method

Select a thoroughly clean and dry pycnometer. Fill the pycnometer with the substance and adjust the temperature of the filled pycnometer to 25º, remove any excess of the substance and weigh. Fill the pycnometer with water, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the specific gravity of the liquid by dividing the weight of the liquid contained in the pycnometer by the weight of water contained, both determined at 25º.

5. Refractive Index

The refractive index \((n)\) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It varies with the wavelength of the light used in its measurement. Unless otherwise prescribed, the refractive index is measured at 25º(±0.5) with reference to the wavelength of the D line of sodium (=589.3 nm). The temperature should be carefully adjusted and maintained since the refractive index varies significantly with temperature. The Abbe refractometer is convenient for most measurements of refractive index but other refractometers of equal or greater accuracy may be used. Commercial refractometers are normally designed for use with white light but are calibrated to give the refractive index in terms of the D line of sodium light.

6. Determination of Peroxide Value

The peroxide value is the number of milli equivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.
Method
(Unless otherwise specified in the individual monograph) Take 5 g of the substance being examined, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5 ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01 M sodium thiosulphate until the yellow colour almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue colour just disappears. Repeat the operation omitting the substance being examined (b ml). The volume of 0.01 M sodium thiosulphate in the blank determination must not exceed 0.1 ml. Calculate the peroxide value from the expression,
\[
\text{Peroxide value} = 10 \left( \frac{a - b}{w} \right)
\]
Where \( w \) = weight, in g, of the substance.

7. Rancidity test (Kreis Test)
The test depends upon the formation of a red colour when oxidized fat is treated with conc. HCl and a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is epihydrin aldehyde. All oxidized fats respond to the Kreis test and the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity.

Procedure
Mix 1 ml of melted fat and 1 ml of conc. HCl in a test tube. Add 1 ml of a 1% solution of phloroglucinol in diethyl ether and mix thoroughly with the fat-acid mixture. A pink colour formation indicates that the fat is slightly oxidized while a red colour indicates that the fat is definitely oxidized.

7. HPTLC Analysis
Sample Preparation
0.5 ml of the oil was taken in a conical flask and was diluted to 10 ml solution with ethyl acetate.

Mobile Phase
Cyclohexane: Ethyl acetate: Formic acid (7:3:0.5, v/v/v).
METHODOLOGY

5 µl of the extract was applied on silica coated TLC plate (60 F254) using Camag’s ATS4 applicator. The plate was developed in previously saturated Twin trough chamber (CAMAG) (10× 10 cm) with the prepared mobile phase. The plate was developed up to 90 mm from the bottom. After development, the plate was photo documented using Camag’s TLC Visualizer under UV λ254 nm and UV λ366 nm. Then the plate was scanned using Camag’s Scanner 4 at λ254 nm (D2 lamp, Absorption mode) and λ366 nm (Hg lamp, Fluorescence mode) respectively & finger print profiles of the extract was performed. Subsequently the plate was dipped in vanillin sulphuric acid solution followed by heating at 105oC till development of the coloured spots. The plate was then photo documented in white light using Camag’s TLC Visualizer & scanned at λ520 nm (W light, Absorption mode).

RESULTS

The results of physicochemical parameters are shown in Table 1.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Physico-chemical Parameter</th>
<th>I</th>
<th>II</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acid value</td>
<td>14.38</td>
<td>13.80</td>
<td>14.09</td>
</tr>
<tr>
<td>2.</td>
<td>Saponification value</td>
<td>78.17</td>
<td>77.11</td>
<td>77.61</td>
</tr>
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<td>3.</td>
<td>Iodine value</td>
<td>102.43</td>
<td>101.36</td>
<td>101.90</td>
</tr>
<tr>
<td>4.</td>
<td>Specific gravity</td>
<td>0.916</td>
<td>0.926</td>
<td>0.921</td>
</tr>
<tr>
<td>5.</td>
<td>Refractive index</td>
<td>1.465</td>
<td>1.465</td>
<td>1.465</td>
</tr>
<tr>
<td>6.</td>
<td>Peroxide value</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>7.</td>
<td>Rancidity</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

The results of HPTLC at wavelength of 254 nm show that there are 8 Phytoconstituents and corresponding ascending order of Rf values starts from 0.38 to 1.13 in which highest concentration of phytoconstituents is found to be 47.68% and its corresponding Rf value is found to be 1.13. (Fig 1, 2 & 3).
The results of HPTLC at wavelength of 254 nm show that there are 18 Phytoconstituents and corresponding ascending order of Rf values starts from 0.13 to 1.30 in which highest concentration of phytoconstituents is found to be 21.87% and its corresponding Rf value is found to be 0.78. (Fig 4, 5 & 6).
The results of HPTLC at wavelength of 520 nm show that there are 8 Phytoconstituents and corresponding ascending order of Rf values starts from 0.15 to 1.13 in which highest...
concentration of phytoconstituents is found to be 28.07% and its corresponding Rf value is found to be 0.51. (Fig 7, 8 & 9).

Figure 7: HPTLC of VKT @ 520 nm.

Figure 8: HPTLC of VKT: Peak table @ 520 nm.

Figure 9: HPTLC of VKT: 3D Chromatogram @ 520 nm.
SUMMARY AND CONCLUSION
In general, Vatha Kesari Thailam (VKT) has acid value of 14.09, Saponification value of 77.61, Iodine Value of 101.90, Specific gravity of 0.921 and Refractive value of 1.465. It is non rancid oil. HPTLC study provides a chromatographic fingerprint of phytochemicals and confirms the identity and purity of medicinal plant raw materials used in VKT. The values obtained after analysis were found to be within permissible limits of a general Tailam (oil preparation) mentioned in Siddha Pharmacopoeia. The obtained values may be taken as standard parameters for future preparations.

ACKNOWLEDGEMENT
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REFERENCES