UNIQUE JOURNAL OF AYURVEDIC AND HERBAL MEDICINES
Research Article

STANDARDIZATION OF VIPADIKAHARA GHRITA TAILA: AN AYURVEDIC MEDICATED OIL FOR COMMON SKIN LESIONS

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Received 09-06-2013; Revised 30-06-2013; Accepted 15-07-2013

ABSTRACT

"Vipadikaharagritataila" was mentioned as the treatment for "Vipadika" and another four types of skin lesions under the treatment component of skin diseases in Ayurvedic text "Caraka Samhita". Though Vipadikahara ghrita taila is recommended for common skin diseases, scientifically controlled techniques were not established to standardize this medicated oil. Therefore, present study was designed to evaluate the physicochemical parameters and develop the TLC fingerprint profiles of Vipadikahara ghrita taila.

Physico- chemical parameters such as specific gravity (0.92 ± 0.00), acid value (3.63 ± 0.43 mg KOH/g), saponification value (441.83 ± 5.97mg/g), peroxide value (0.95±0.04 Meq/Kg) and iodine value (53.50±1.25 g I_2/100g) were determined. Thin Layer Chromatography fingerprint profiles were developed for Vipadikahara ghrita taila and standard mixture of plant materials. The results obtained for physico-chemical parameters and R_f values of TLC fingerprint profiles may be used as tools to standardize Vipadikahara ghrita taila.

Keywords: Standardization, Skin diseases, Ayurvedic medicated oil, Vipadikahara ghrita taila

INTRODUCTION

Medicated oils occupy an important section of Ayurveda Pharmacology and this holistic health care system prescribes usage of different medicated oils for the body for external as well as internal usage to provide health benefits. Numbers of medicated oils were mentioned in Ayurvedic original texts for various types of skin diseases. Some of them contain large number of ingredients and have to follow difficult preparation methods. If we can find effective, easy prepared, inexpensive medicated oil with less number of ingredients, it will be benefit to human being.

Prevalence of skin disease was 47.6% in suburban Sri Lanka, the only available measures of disease frequency related to skin disorders are from hospital-based clinic studies. There were marked differences between community prevalence and hospital dermatology clinic attendance data for a number of skin diseases[1]. Vipadikahara ghrita taila is an easy prepared, fragrant medicated oil which can be used externally to treat five types of skin diseases according to Ayurvedic authentic text Caraka Samhita[2]. Medicinal plants and other ingredients of "Vipadikahara ghrita taila" are: *Leptadenia reticulate* (Retz) Wight and Arn**.** (F: Asclepiadaceae)*, Rubia cordifolia* Linn Syst. (F: Rubiaceae)*, Berberis aristata* DC. Syst. (F: Berberidaceae), *Mallotus phillipinensis* Muell.Arg. (F: Euphorbiaceae), Cow's milk, Bee's wax, Resin of *Shorea robusta* Geartn (F: Dipterocarpeae), oils from sesame (*Sesamum indicum* Linn) seeds (F: Pedaliaceae) and Cow's ghee.

Moreover, "Vipadikahara ghrita taila" was mentioned as the treatment for "Vipadika" and another four types of skin lesions under the treatment component of skin diseases in Ayurvedic text "Caraka Samhita". "Vipadika" is a very common type of skin disease in Sri Lanka. Normally, regular linear cut wounds up to epidermis can be seen. Further, the fissures of the palms and plantar surfaces become very painful with roughness, burning sensation, and can cause bleeding because those lines go deep into the dermis. Though Vipadikahara ghrita taila is recommended for common skin diseases, scientifically controlled techniques were not established to standardize this medicated oil. Therefore, present study was designed to evaluate the physicochemical

parameters and develop the TLC fingerprint profiles of Vipadikahara ghrita taila.

MATERIALS AND METHODS

Plant materials

The herbarium sheets of *R. cordifolia* and *M. phillipinensis* were authenticated by the Senior Scientist, Botany Section, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka. Furthermore, *L. reticulate* and *B. aristata* were authenticated by the Senior Scientist, Vidyaratnam Foundation (Research and Development Section), Thrissur, Kerala, India.

Preparation of Vipadikahara ghrita taila

Pharmacognostically pure and authentic four medicinal plants, cow's milk, bee's wax, resin of *S. robusta,* sesame oil and cow's ghee were used to prepare Vipadikahara ghrita taila. Preparation of the standard sample of oil was done at the Pharmacy, Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka according to the oil preparing method described in Ayurveda pharmacopoeia. In brief, dirt free plant materials were mixed with water and the mixture was heated using mild flame until the volume of water reduced to one fourth of the original volume. Then cow milk was added and allowed to reduce the volume further. After that, mixture was filtered through a muslin cloth, filtrate was added to a mixture of sesame oil and ghee and boiled until the oil remained. Finally, small amount of bee's wax and resin of *S. robusta* were added. Plant ingredients of Vipadikahara ghrita taila were mentioned in Table 1.

Evaluation of Physicochemical parameters

Physicochemical parameters such as colour, smell, appearance, specific gravity, saponification value, peroxide value, acid value and iodine value were determined in Vipadikahara ghrita taila according to the standard techniques.

Determination of Specific gravity

Firstly empty specific gravity bottle was weighed, and then filled with Vipadikahara ghrita taila and weighed again. After that, specific gravity bottle was well cleaned, dried, filled with distilled water and weighed. The difference in weights was divided by the weight of an equal volume of water to give the specific gravity of Vipadikahara ghrita taila.

Determination of Acid value

In brief, oil sample was accurately weighed and added into a 200 mL conical flask. Then 100 mL of freshly neutralized hot ethyl alcohol and 1 mL phenolphthalein were added to the above mixture, boiled for 5 min. and titrated while as hot as possible with standard 0.25 N KOH solution as per SLS[3].

Calculation:

$$\text{Acid value} = \frac{56.1 \times V \times N}{W}$$

Where

V – Volume in mL of standard KOH solution used

N – Normality of standard potassium Hydroxide solution

W – Weight in g of the material taken for the test

Determination of Saponification value

In brief, oil sample was accurately weighed (about 1.5g) and added to a conical flask. Then alcoholic potassium hydroxide (25 mL) was added and connected the reflux air condenser to the flask. The flask was heated on the water bath for a 1 h. When the flask and condenser were cooled, inside of the condenser was washed down with 10 mL of hot ethyl alcohol. Finally, 1 mL of phenolphthalein was added and titrated with standard hydrochloric acid. A blank titration was done at the same time[4].

Calculation:

$$\text{Saponification value} = \frac{56.1\ (B - S)\ N}{W}$$

B = Volume in mL of standard hydrochloric acid required for the blank

S = Volume in mL of standard hydrochloric acid required for the sample

N= Normality of the standard hydrochloric acid

W= Weight in g of the material taken for the test

Determination of peroxide value

In brief, oil sample was accurately weighed (5 g) into a glass stopper conical flask and then added 30 mL of acetic acid – chloroform solution, swirled the flask until the sample is dissolved and added 0.5 mL of saturated potassium iodide solution. Then allowed the solution to stand exactly one minute with occasional shaking and then added 30 mL of distilled water. Finally, titrated with 0.1 N sodium thiosulphate solution with constant and vigorous shaking. Continued the titration until the yellow colour almost disappears and added 0.5 mL of starch solution and continue titration till the blue colour just disappears as described in the SLS[3].

Calculation:

$$\text{Peroxide Value} = \frac{(S - B) \times N \times 1000}{g}$$

Where

S = Volume in ml of sodium thiosulphate solution used up by the sample,

B = Volume in ml of the sodium thiosulphate solution used up in the blank determination,

N = Normality of the sodium thiosulphate solution

g = Weight in g of the sample

Determination of iodine value

Filtered pure oil sample was accurately weighed (5 g) into absolutely clean and dry 500 mL iodine flask. Then 25 mL of carbon tetrachloride was added and dissolved the contents. Then 25 mL of the Wijs solution (the weight of the sample was an excess of 50% of Wijs solution actually needed) and replaced the glass stopper after wetting with potassium iodide solution; swirled for mixing, and allowed to stand in dark for 1 h. A blank test was carried out under similar experimental conditions. Then potassium iodide solution (15 mL) and water (100 mL) were added. Finally, liberated iodine was titrated with standard sodium thiosulphate solution. Swirled the contents of the bottle continuously until colour of the solution was straw colour. Then added the starch solution (1 mL) and continued the titration until the blue colour disappeared[4].

$$\text{Iodine Value} = \frac{12.69\ (B\text{-}S)\ N}{W}$$

B= Volume of mL of standard sodium thiosulphate solution required for the blank

S= Volume of mL of standard sodium thiosulphate solution required for the sample
N= Normality of the standard sodium thiosulphate solution
W= Weight in g of the material taken for the test.

Development of Thin Layer Chromatography (TLC) fingerprints for (a) plant ingredients and (b) medicated oil

(a) Water extract of four plant ingredients

Each plant ingredient (2.5 g/plant) was weighted into a conical flask and 200 mL of water was added. Then it was boiled using the hot plate (Fissher stirring hot plate, 004N0035, USA) until the volume reduced to 100 mL. Then the extract was filtered, filtrate was transferred to the separatory funnel. After that sequential fractionation was carried out using dichloromethane and ethyl acetate. Finally, dichloromethane fraction and ethyl acetate fraction were concentrated using a rotovapour (Buchi, B-480) separately and spotted on TLC plates.

(b) Medicated oil

In brief, oil sample was accurately weighed (5.0 g) into a round bottom flask and distilled water (100 mL) was added and refluxed for 1 h. Using a separatory funnel water layer was separated and sequential fractionation was carried out using dichloromethane and ethyl acetate. Finally, dichloromethane fraction and ethyl acetate fraction were concentrated using a rotovapour (Buchi, B-480) separately and spotted on TLC plates. TLC fingerprint profile were determined using following conditions:

Solvent system for plant ingredients :

For dichloromethane fraction

Methanol: Cyclohexane: Dichloromethane (0.2: 1.0: 3.8 v/v/v)

For ethyl acetate fraction

Methanol: Cyclohexane: Dichloromethane (0.25: 1.5: 3.75 v/v/v)

Solvent system for oil:

For dichloromethane fraction

Methanol: Cyclohexane : Dichloromethane (0.2 :1.0 : 3.8 v/v/v)

For ethyl acetate fraction

Methanol: Cyclohexane:Dichloromethane (0.25 :1.5: 3.75 v/v/v)

Absorbent

Silica gel-GF_{254} pre- coated plate

Detection

Direct visualization: Under UV (λ at 254 nm &366 nm)

: Vanillin sulphuric acid was sprayed to the TLC plate and heated at 105 ^{o}C for 5 min.

Scanning: Densitometer (CS – 9301PC, Shimadzu, Japan at 254 nm) (Before spraying)

RESULTS AND DISCUSSION

The herbal preparations have to be standardized in order to get the optimal concentrations of known active constituents, and in preserving their activities. The physicochemical parameters of Vipadikahara ghrita taila are shown in Table 2. Normally, oils give different characteristic color and odour relative to the herbs and other materials which were used to prepare the oil. In this medicated oil, red colour is given due to *R. cordifolia* and red glands and hairs of *M. phillipinensis*. The characteristic odor is due to the ghee and sesame oil which were used as the oil base. The saponification value of Vipadikahara ghrita taila was 441.83± 5.97 mg/g. This value is outside of the range (188- 196 mg/g) for most oils of plant origin[5]. Saponification value is the number of milligram of potassium hydroxide required neutralizing the fatty acids[6]. It is a measure of the average molecular weight of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. If more base are required to saponify nitogen grams of fat then there are more moles of the fat the chain lengths are relatively small[7].

The acid value is the mass of potassium hydroxide in milligrams that is required to neutralize one gram of chemical substance. Acid value is used to quantify the amount of acid present in an oil sample. It is the quantity of base, expressed in milligrams of potassium hydroxide that is required to neutralize the acidic constituents in one gram of the sample[8,9]. Acid values are used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat[10]. According to the results, acid value of Vipadikahara ghrita taila was 3.63 ± 0.43 mg KOH/g. The peroxide value is a measure of the active oxygen in the oil and the potential to go rancid. High starting levels of peroxide values are a bad sign. According to the SLS 1341 : 2008 standard, upper limit of the peroxide value of a oil is 10 Meq/Kg. Peroxide value of the Vipadikahara ghrita taila was 0.95±0.04 Meq/kg. In general, peroxide levels higher than 10 may mean less stable oil with a shorter shelf life. Factors that increase the peroxide value include high temperature, visible light and oxygen. Contact with metal surfaces, such as copper, can also catalyse oxidation of the oil. Oil should be stored in cool, inert vessels, such as stainless steel or glass, away from the light[11].The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation (Danlami and David, 2012). According to the present study, iodine value of Vipadikahara ghrita taila oil was 53.50±1.25 g I_2/100g. TLC fingerprint profiles were viewed under UV at 254 nm, UV 366 nm and also viewed after spraying the spray reagent vanillin sulphuric acid and recorded the R_f values (Table 3). TLC fingerprint profile of the Vipadikahara ghrita taila was similar to that of standard mixture of plant ingredients.

CONCLUSION

In conclusion, these quality-control parameters and the developed TLC fingerprints may be considered as tools for assistance for scientific organizations and manufacturers in developing standards for Vipadikahara ghrita taila.

ACKNOWLEDGEMENT

National Centre for Advanced Studies for Humanities and Social Sciences, Ward Place, Colombo ,7 Sri Lanka is acknowledged for providing financial assistance.

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Table 1: Ingredients of Vipadikahara ghrita taila

Ingredients	Part of the plant
Leptadenia reticulate (Retz) Wight and Arn.	Leaves and stem
Rubia cordifolia Linn Syst.	Stem
Berberis aristata DC.(IO)	Stem
Mallotus phillipinensis Muell.Arg.	Glands and hairs (red powder)
Shorea robusta Geartn.	Resin
Sesamum indicum Linn	Seed oil
Cow's Milk and Ghee	

Table 2: Physicochemical parameters of Vipadikahara ghrita taila

	Physicochemical parameters	Results
1.	Colour	Reddish brown
2.	Odor	Fragrant
3.	Appearance	Viscous
4.	Touch	Oily
5.	Clarity	Not clear in room temperature and clear when melting
6.	Specific gravity	0.92 ± 0.00
7.	Acid value (mg KOH/g)	3.63 ± 0.43
8.	Saponification value mg/g	441.83 ± 5.97
9.	Peroxide value Meq/Kg	0.95±0.04
10.	Iodine value (g I_2/100g)	53.50±1.25

Table 3: R_f values for Vipadikahara ghrita taila and standard mixture of plant ingredients

	R_f values	
	Dichloromethane fraction	**Ethyl acetate fraction**
Vipadikahara ghrita taila	0.06, 0.37, 0.50, 0.54, 0.72, 0.91	0.06, 0.10, 0.25,0.45, 0.52, 0.64
Standard mixture of plant ingredients	0.06,0.12, 0.20, 0.42, 0.50, 0.91	0.06, 0.10,0.52, 0.64, 0.87, 0.95

Source of support: Nil, Conflict of interest: None Declared