

Standardization of a Substitute Oil for Vipadikahara Gritataila which Used for Skin Diseases

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ABSTRACT

Background: “Vipadikahara gritataila” was mentioned as a treatment especially for common skin lesion “Vipadika” and another four types of skin lesions under the treatment component of skin diseases in Ayurvedic text “Caraka Samhita”. Though it is an effective treatment, two important plant materials are not available in Sri Lanka to prepare this medicated oil. Present study was designed to (1) Prepare the substitute oil using two newly identified substitute plant materials *Wattakaka volubilis* and *Berberis ceylanica* (2) Evaluate the physicochemical parameters and develop the Thin Layer Chromatography (TLC) fingerprint profiles. **Materials and Methods:** Preliminary physicochemical parameters such as colour, smell, appearance, specific gravity, saponification value, peroxide value, acid value and iodine value were determined in substitute oil according to the standard techniques. Development of TLC fingerprints for (a) Plant ingredients and (b) Medicated oil were done using dichloromethane and ethylacetate fraction. **Results:** substitute oil was almost same as Vipadikahara gritataila in terms of physico-chemical parameters such as specific gravity (0.9100 ± 0.00), acid value (4.49 ± 0.33 mg KOH g^{-1}), saponification value (474.04 ± 6.67 mg g^{-1}), peroxide value (1.47 ± 0.0 Meq kg^{-1}) and iodine value (57 ± 1.06 g I_2 $100 g^{-1}$). The results obtained for physico-chemical parameters and R_f values of TLC fingerprint profiles may be used as tools to standardize substitute oil. **Conclusion:** This study revealed that the prepared oil has very similar properties to Vipadikahara gritataila and it can be used as substitute oil for recommended skin lesions.

Key words: Substitute oil, vipadikahara gritataila, ayurvedic medicated oil, standardization, *Wattakaka volubilis*, *Berberis ceylanica*

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INTRODUCTION

Ayurveda, a holistic health care system prescribes usage of different medicated oils for the body, for external as well as internal usage to provide health benefits. Medicated oils are one of the main items among the medicines which helped to heal the diseases in Ayurvedic practice. Especially for skin ailments, oils are helped to reduce the roughness of the skin, itching and burning sensation and cure the skin rashes. Some medicated oils are very difficult to prepare as they have large number of ingredients and difficult methods of preparation.

Medicated oil vipadikahara gritataila is an effective, fragrant preparation which can be used externally to treat five types of skin diseases according to Ayurvedic authentic text Caraka samhita (Senagupta,1855). Medicinal plants and other ingredients of “Vipadikahara gritataila” are *Leptadenia reticulata* (Retz) Wight and Arn. (*L.reticulata*) family: Asclepiadaceae, *Rubia cordifolia* Linn Syst. (*R. cordifolia*) family: Rubiaceae, *Berberis aristata* DC. Syst (*B. aristata*), family: Berberidaceae, *Mallotus philippinensis* Muell. Arg. (*M. philippinensis*) family: Euphorbiaceae and Cow’s milk, Bee’s wax, Resin of *Shorea robusta* Geartn, family: Dipterocarpeae. Two oils: Sesame (oil of *Sesamum indicum* Linn seeds), family: Pedaliaceae and cow’s ghee were used as the base of the oil.

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Though it contains less number of plant materials, *L. reticulata* and *B. aristata* are not available in Sri Lanka. Therefore, finding suitable substitutes for those two plants are very important and cost effective. *Wattakaka volubilis* (*W.volubilis*) Family: Asclepiadaceae is a common medicinal plant, frequently used by traditional practitioners of Sri Lanka especially for nervous and respiratory disorders (Illiyakperuma, 1879) and Kerala used its leaves to treat inflammatory and painful conditions (Vishnusithan and Kamaraj, 2012). In the field of Ayurvedic medicine, the plant *B. aristata* was cited in different medicinal preparations specially used for skin ailments, menorrhagia, diarrhoea, jaundice, painful micturition due to acid urine (Kirtikar and Basu, 1975).

W. volubilis showed very similar properties to *L. reticulata* according to phytochemical, physicochemical and TLC fingerprint profiles (Hewageegana *et al.*, 2012). *Berberis ceylanica* (*B. ceylanica*), family: Berberidaceae origin in Sri Lanka was shown very similar properties *B. aristata* in physico-chemically and as well as phytochemically (Nawarathna *et al.*, 2010). Therefore, substitute oil was formulated using freely available above mentioned two medicinal plants: *W. volubilis* (instead of *L. reticulata*) and *B. ceylanica* (instead of *B. aristata*). Thus, it is needed and important to develop a scientific accepted method to ascertain the standard and purity of the above newly prepared substitute oil at first time in Sri Lanka.

According to that, the aim of the present study was to evaluate the physico-chemical standards and TLC fingerprint profiles of substitute oil for Vipadikahara gritataila.

MATERIALS AND METHODS

Pharmacognostically pure and authentic ingredients were used for the preparation of substitute oil. The herbarium sheets of *R. cordifolia*, *M. philippinensis*, *W. volubilis* and *B. ceylanica* were authenticated by the Senior Scientist and Voucher specimens were deposited Botany Section, Bandaranayaka Memorial Ayurveda Research Institute, Navinna, Maharagama, Sri Lanka.

Preparation of substitute oil: A preparation of the standard sample of the oil was done at Pharmacy, Institute of Indigenous Medicine, University of Colombo, Rajagiriya according to the basics of Ayurveda pharmacopoeia in Sri Lanka. 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; mixture of sesame oil and ghee was added to the filtrate and boiled until the oil remained. Finally, small amount of bee's wax and resin of *S. robusta* were added. Ingredients of substitute oil were mentioned in Table 1.

Table 1: Ingredients of substitute oil for Vipadikahara gritataila

Ingredients of substitute oil	Part of the plant
<i>Wattakaka volubilis</i>	Leaves and stem
<i>Berberis ceylanica</i> Schneider.	Stem
<i>Rubia cordifolia</i> Linn Syst	Stem
<i>Mallotus philippinensis</i> Muell.Arg.	Glands and hairs (red powder)
<i>Shorea robusta</i> Geartn.	Resin
<i>Sesamum indicum</i> Linn	Seed oil
Cow's Milk and Ghee	

Evaluation of physicochemical parameters:

Preliminary physicochemical parameters such as colour, smell, appearance, specific gravity, saponification value, peroxide value, acid value and iodine value were determined in substitute oil according to the standard techniques.

Determination of specific gravity: Firstly empty specific gravity bottle was weighed and then filled with substitute oil 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 substitute oil.

Determination of acid value and peroxide value:

Acid and peroxide values were evaluated according to SLS standard (Sri Lanka standard, 2008).

Determination of saponification value and iodine value:

Saponification and iodine values were evaluated according to Indian standards (Indian Standard, 1976).

Development of Thin Layer Chromatography (TLC) fingerprints for (a) Plant ingredients and (b) Medicated oil

Water extract of four plant ingredients: Each plant ingredient (2.5 g plant⁻¹) was weighted into a conical flask and 200 mL of water was added. Then it was boiled using the hot plate (Fisher 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.

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 was 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-GF₂₅₄ pre- coated plate

Detection**Direct visualization:** Under UV

(λ at 254 nm and 366 nm)

- Vanillin sulphuric acid was sprayed to the TLC plate and heated at 105°C for 5 min

Scanning: Densitometer (CS-9301PC, Shimadzu, Japan at 254 nm) (before spraying)

RESULTS AND DISCUSSION

Physicochemical parameters of oil are summarized in Table 2. R_f values for Substitute oil and standard mixture of plant ingredients are in Table 3. Normally, oils give different characteristic color and odour relative to the herbs and other materials which were used to prepare the oil. In Vipadikahara gritataila, red colour is given due to *R. cordifolia* and red glands and hairs of *M. philippinensis* (Hewageegana *et al.*, 2013). According to the results of this study, most of the parameters such as colour, smell, appearance, touch, clarity and specific gravity of the substitute oil was similar to that of Ayurvedic medicated oil, Vipadikahara gritataila which used to treat common skin diseases. Moreover, the characteristic odor present in both Vipadikahara gritataila and substitute oil was due to the ghee and sesame oil which were used as the oil base. According to Table 2, physico-chemical parameters [peroxide value (1.47 ± 0.04 Meq kg^{-1}), iodine value (57 ± 1.06 g I_2 100 g^{-1}) and acid value (4.49 ± 0.33 mg KOH g^{-1})] of the substitute oil were not significantly different from that of Vipadikahara gritataila.

Peroxide value is a measure of the active oxygen in the oil and high starting levels of peroxide values are a bad sign. According to the SLS 1341: 2008 standard, upper limit of the peroxide value of oil is 10 Meq kg^{-1} . In general, peroxide levels higher than 10 may mean less

Table 2: Physicochemical parameters of substitute oil for Vipadikahara gritataila

Parameter	Substitute oil
Colour	Reddish brown
Smell	Fragrant
Appearance	Viscous
Touch	Oily
Clarity	Not clear in room temperature and clear when melting
Specific gravity	0.91 ± 0.00
Acid value (mg KOH g^{-1})	4.49 ± 0.33
Saponification value (mg g^{-1})	474.04 ± 6.67
Peroxide value (Meq kg^{-1})	1.47 ± 0.0
Iodine value (g 100 g^{-1})	57 ± 1.06

Table 3: R_f values of substitute oil for Vipadikahara gritataila and its standard mixture of plant ingredients

	R_f values	
	Dichloromethane fraction	Ethyl acetate fraction
Substitute oil	0.23, 0.28, 0.36, 0.46, 0.53	0.42, 0.49, 0.55, 0.64, 0.77
Standard mixture of plant ingredients	0.07, 0.11, 0.13, 0.18, 0.38, 0.47, 0.53, 0.54, 0.71, 0.87	0.07, 0.11, 0.18, 0.2, 0.23, 0.3, 0.51

stable oil with a shorter shelf life. In the present study, peroxide value of the substitute oil was well below the 10 Meq kg^{-1} . 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). However, iodine value of the substitute oil was at a moderate level (57 ± 1.06 g I_2 100 g^{-1}). In oils, saponification value is the number of milligram of potassium hydroxide required to neutralizing the fatty acids (Kumara and Nishteswar, 2011). This value is outside of the range (188-196 mg g^{-1}) for most oils of plant origin (Pearson, 1976). 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 nitrogen grams of fat then there are more moles of the fat the chain lengths are relatively small (Mahale *et al.*, 2011). In the present study, saponification value of the substitute oil (474.04 ± 6.67 mg g^{-1}) was significantly higher than that of Vipadikahara gritataila (441.83 ± 5.97 mg g^{-1}) (Hewageegana *et al.*, 2013).

TLC fingerprint profiles were viewed under UV (at 254 nm, UV 366 nm) and after spraying the spray reagent, vanillin sulphuric acid. Further, R_f values of the TLC fingerprint profiles (before and after spraying the spray reagent) were presented in Table 3. When compared the R_f values of the substitute oil and the standard mixture of plant ingredients it was evident that all the added plant ingredients were present in the substitute oil (Table 3).

CONCLUSION

In conclusion, this study reveals that two medicinal plants *W.volubilis* and *B. ceylanica* can be used as the substitute for preparing effective medicated oil. Moreover, quality-control parameters and developed TLC fingerprints may be considered as tools for developing standard substitute oil for Vipadikahara gritataila.

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