Comparative Preliminary Standardization of *Siddha* Medicated Oil: *Thalangai Ennai*, a Poly Herbal Formulation

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Abstract- A poly herbal *Siddha* formulation, *Thalangai ennai* (T.E) was prepared as per the *Siddha* Literature and used as relieving rheumatic pains, impaired movement of limbs, and all kind of joint disorders, etc. The physicochemical standards available for the standardization of *Siddha* medicated oils are insufficient. This article reports on comparative preliminary standardization of T.E of Drug Unit (DUT) and Marketed formulation (MFT) have been standardized on the basis of organoleptic characters, physicochemical properties and analysis of Thin Layer Chromatography. Specific gravity and pH value for DUT was found to be 0.9185 and 4.61; in case of MFT these were found to be 0.9173 and 4.97 at room temperature respectively. The TLC finger prints of both the formulations were revealed fifteen spots in the pure dichloromethane solvent. The results obtained with these both the formulations were found to be comparable and variations were not remarkable. Though the analytical values can be used as reference standards for the quality assurance of T.E, these values mostly related to the purity of the sesame oil (which is used as a base), the qualitative and quantitative estimation of each single ingredient of T.E requires further research work in future.

Index Terms- Thalangai ennai, Poly herbal formulation, Medicated Oil, Siddha Medicine, Standardization

I. INTRODUCTION

With the 'retain to nature' call in both, the developed countries as well as developing countries, there is an increase in number of people switching over to the alternative system of medicine and thus it is essential that people get relevant medicines [1]. *Siddha* Medicine is a one of the traditional medicine [2] and practiced by Tamil people of Northern and Eastern province in Sri Lanka. An attempt is made to standardize *Siddha* medicated oil.

Effective management of *vātā* disorders such as rheumatic pain, inflammation, bone degenerative changes and impaired movement of limbs are the important tasks in the field of treatment. *Siddhars* described *vātā* disorders of different origin and external application is most useful for their management [3, 4].Among them *Ennai* (Medicated oil) is one of the medicaments prescribed by them [5, 6, 7]. T.E is one of the *Siddha* medicated oil mentioned in the *Siddha* Literature [8] and it is prepared at Government Indigenous Medicine Drug Unit in Jaffna and supplied to all Government District Ayurvedic Hospital, Rural Ayurvedic Hospitals and Central Ayurvedic Dispensaries in Jaffna District. A marketed formulation of T.E (Everest product) is available in Jaffna District. The ingredients used in T.E are juice of the *thalangai* (*Pandanus tectorius*), sesame oil, milk, water, rock salt and seventeen different herbal powdered materials [9] with that it is one of the most effective oil for external applications to the rheumatic conditions.

The physicochemical standards available for the standardization of *Siddha* medicated oils are insufficient. The analytical values available in the pharmacopoeial standards for *siddha* formulations are not finger print standards for each of oil [10]. The aim of the present study was to fixed preliminary reference standards of the T.E. This article reports on comparative preliminary standardization of T.E (preparation of Drug Unit, Indigenous Medicine and the marketed formulation) have been standardized on the basis of physicochemical properties, organoleptic characters, and Thin Layer Chromatography (TLC) finger print analysis.

I. Methodology

Marketed samples for external medicine

T.E was purchased from a reputed vendor of herbal Medical products, *Everest Marunthakam*, *Usan*, *Mirusuvil* (Registered at Sri Lanka Ayurveda Medical Council). The marketed formulation of T.E (MFT) was standardized based on their organoleptic characters (physical characteristic), physicochemical properties (pH, specific gravity) and thin layer chromatography (TLC) according to standard procedures and compared with the T.E of Drug Unit of Indigenous Medicine, Jaffna (DUT).

Ingredients and preparation method of Thalangai ennai

Botanically pure and authentic ingredients were used in the preparation of T.E. Table No. 1 shows the ingredients and its quantity for preparation of T.E in accordance with the guidelines stated in the *Siddha* Literature [8, 9]. From 1 to 17 dried raw materials are ground to get coarse powder and mixed with 10 times by volume of water (16 L). Then continued boiling to reduce the volume to one eighth (2 L) and the decoction is strained using a muslin cloth to obtain the aqueous decoction. Juice of the *thalangai*, sesame oil, milk (2:1:1) are taken in a vessel and heated for some time; mixed the aqueous decoction together. This mixture is boiled on mild fire with stirring and boiling continued till all the water evaporates and this mixture turn as oily (waxy like). After cooled 10 minutes, the powdered rock salt mixed with the oil.

Table1: Ingredients and its quantity for preparation of *Thalangai ennai* [11, 12, 13, & 14]

No	Local name/	Scientific name	Family name	Part used	Quantity
01	Nannari	*Hemidesmus indicus*	Asclepiadaceae	Root	10 g
02	Villamichu	*Plectranthus zeylanicus*	Labiatae	Root	10 g
03	Vetti	*Andropogon muricatus*	Gramineae	Root	10 g
04	Sittamutti	*Pavonia zeylanica*	Malvaceae	Root	10 g
05	Peramutti	*Pavonia odorata*	Malvaceae	Root	10 g
06	Tevadaru	*Cedrus deodara*	Coniferae	Root	10 g
07	Jatamashi	*Nardostachys jatamansi*	Velerianaceae	Root	10 g
08	Kacholum	*kaempferia galanga*	Zingiberaceae	Root	10 g
09	Mara manjal	*Coscinum fenestratum*	Menispermaceae	Stem	10 g
10	Sandanam	*Santalum album*	Santalaceae	Stem	10 g
11	Kottam	*Costus speciosus*	Scitaminaceae	Stem	10 g
12	Kundurukkan	Boswellia serrata	Burseraceae	Seed	10 g
13	Adi-maduram	Glycyrrhizae glabra	Papilionaceae	Stem	10 g
14	Satakuppi	Peucedanum graveolens	Umbelliferae	Resin	10 g
15	Chirttilam/elakaya	Eletteria cardamomum	Zingiberaceae	Seed	10 g
16	Patchchilai	*Zanthochymus piotorlins*	-	Leaves	10 g
17	Korai-kizangu	*Cyperus rotundus*	Cyperaceae	Rhizome	10 g
18	Thazhai/ thazaai	*Pandanus tectorius*	Pandanaceae	Juice	1250 ml
19	Rock salt	NaCl impura		Salt	10 g
20	Sesame oil	*Sesamum indicum*	Pedaliaceae	Seed oil	625 ml
21	Cow's milk	*Bos indicus inn*		milk	625 ml
22	Water	-	-	-	16000 ml

Organoleptic evaluation

Both samples of oil (T.E) and its water and dichloromethane extracts were subjected to the organoleptic characterization such as appearance, touch, colour, clarity and odour and compared with the values of Sesame or Gingerly oil (Which is used as a base in preparing these oils) separately. The organoleptic characters of the samples were evaluated based on the method described by *Siddiqui et al.*, 1995 [15].

Physicochemical investigations

Both samples of oil (T.E) were subjected for determination of preliminary physicochemical parameters such as pH value and specific gravity.

Determination of pH range

The pH of both samples of oil (T.E) was determined using standard simple glass electrode pH meter. The individual oil was weighed to about 40ml in separate beakers and the pH of the formulations was determined using a pH meter (Consort) at room temperature (29 ^{0}C).

Determination of specific gravity

Clean, dry and weighed the specific gravity bottle along with the stopper (Weight 'A'). Then filled the specific gravity bottle with freshly distilled water and inserted the stopper firmly. Kept it in a room temperature and weighed it (Weight 'B'). Finally, filled the specific gravity bottle with the oil samples and weighed separately (Weight 'C') [16]. Calculated and entered the average of the two results.

Specific gravity (oil) = $\frac{C-A}{B-A}$

Development of Thin-layer chromatography (TLC) fingerprints of Thalangai ennai

Thin Layer Chromatography (TLC) finger print of the T.E was studied after dissolving the oil in water and dichloromethane.

Prior to testing, prepared a water and dichloromethane extract of the oil to examined, used a rapid extraction process, as followed. Place about 50 ml T.E and fume chips in an accurately weighed, glass-stoppered round bottom flask. For the water extraction, 20 ml water was added to the flask and weighed to obtain the total weight including the flask. Then extraction was done by using reflux condenser for 2hours on heating mantle (Electro thermal). Then cooled and transferred to the Separator funnel system. After 20 minutes, the water extract was separated in to the clean small flask and kept tightly closed with lid. Further 20 ml water added to the residue in the round bottom flask and water extraction was done again as the same procedure.

After that, the whole separated water extract (30 ml) poured in to the separator funnel system. Then 10 ml dichloromethane added to the water extract and mixed well. After 20 minutes, the dichloromethane extract was separated in to the accurately weighed, small round bottom flask and kept tightly closed with lid. Further 10 ml dichloromethane added to the residue in the separator funnel system and dichloromethane extract was separated again as the same procedure. Then this procedure repeated again. Totally, dichloromethane extract separation was done for three times.

Whole dichloromethane extract poured in to the round bottom flask and evaporated on a rotatory evaporator (Buchi) for just as long as was required to remove the solvent, and re-dissolved the residue in a small volume of dichloromethane (3 ml). Finally, the whole extract was collected in clean stoppered glass test tube and used for spotting the chromatographic plates.

Five µl of dichloromethane extract of each T.E (MFT&DUT) was spotted on to TLC plates (8.5 x 5.3 cm) coated with silica gel (pre-coated, GF_{254}) and separated using a same solvent systems. Although the separation of the extracts occurred in the solvent system comprised of hexane: dichloromethane: 1% methanol in dichloromethane (1:4:5 v/ v) as the mobile phase, the best separation of the extracts occurred in the solvent system comprised of pure dichloromethane as the mobile phase. After development visible spots were not found for each oil extract. Numbers of spots were observed under day light and UV light (254 & 366 nm). Visualization was attempted by spraying with vanillin sulphate reagent for each oil extract and heating the plate for 5-10 minute (100-105°C). The colour and Rf values of the spots were recorded carefully and the chromatogram was documented by graphical copying (WHO, 1998).

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II. Results

Both samples values were recorded and compared with values of sesame oil (which is used as a base in preparing this oil).

Organoleptic characters and physicochemical properties

Table 2, summarizes the organoleptic properties, pH values and Specific gravity of the Marketed formulation (MFT) and Drug Unit of Indigenous Medicine (DUT) T.E with Sesame oil at room temperature and Table 3 summarizes the organoleptic properties of its water and dichloromethane extracts.

As seen in Table 2and figure 1, both Brands of the T.E had similar organoleptic properties except for the colour of the each oil compared with Sesame oil. The pH and specific gravity of these brands were showed not remarkable difference between them.

Table2: Analytical parameters of *Thalangai Ennai* MFT & DUT with Sesame oil

Parameters	***Thalangai ennai* (MFT)**	***Thalangai ennai* (DUT)**	**Sesame Oil**
Appearance	Viscous	Viscous	Viscous
Touch	Oily	Oily	Oily
Colour	Red	Golden brown	Brown
Clarity	Clear	Clear	Clear
Odour	Characteristic	Characteristic	Characteristic
pH value (29^0C)	4.97	4.61	4.89
Specific gravity	0.9173	0.9185	0.923

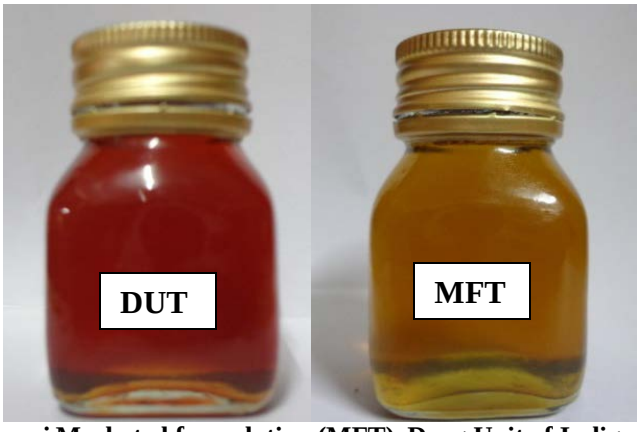

Figure1: *Thalangai ennai* Marketed formulation (MFT), Drug Unit of Indigenous Medicine (DUT)

Table 3: Organoleptic properties of the water, and dichloromethane extracts of *Thalangai ennai* MFT & DUT

Parameters	MFT		DUT	
	Water extract	Dichloromethane extract	Water extract	Dichloromethane extract
Appearance	Liquid	Liquid	Liquid	Liquid
Touch	Liquid	Liquid	Liquid	Liquid

Colour	Colourless	Colourless	Pale yellow	Light yellow
Clarity	Clear	Clear	Clear	Clear
Odour	Characteristic	Characteristic	Characteristic	Characteristic

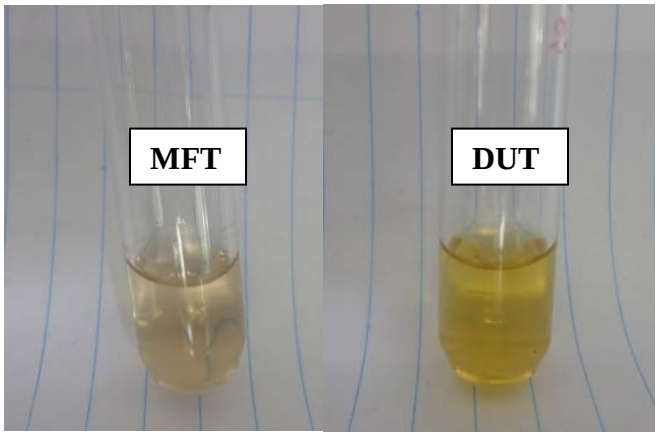

Figure 2: *Thalangai ennai* Marketed formulation (MFT), and Drug Unit of Indigenous Medicine (DUT)

3.2 Thin Layer Chromatography (TLC) finger print analysis

The numbers of different solvent systems were employed to generate fingerprint profiles for these oils. The separation of the oil extracts occurred in the solvent system comprised of Hexane: Dichloromethane: 1% Methanol in Dichloromethane (1: 4: 5 v/ v) and pure Dichloromethane solvent systems as the mobile phase.

Table 4 and Figure 3 summarizes the Rf values and colour of spots visible in the TLC profiles of the Dichloromethane extract of different brands of T.E in Hexane: Dichloromethane: 1% Methanol in Dichloromethane (1: 4: 5 v/ v) solvent system. Table 5 and Figure 4 summarize the Rf values and colour of spots visible in the TLC profiles of the Dichloromethane extract of different brands of T.E in pure Dichloromethane solvent system.

Table 4: TLC analysis of different brands of *Thalangai Ennai*

Rf values and colour of spots	
MFT	DUT
0.017 $^{\$}$ Br.	0.017* Br.
0.067$^{\$}$ Br.	0.07* Br.
0.17* Gn.	0.17* Br.
0.27$^{\#}$ Gn.	0.22$^{\$}$ Gn.
0.40$^{\$}$ Pk.	0.28* Gn.
0.47* Pk.	0.37$^{\#}$ Pk.
0.62$^{\$}$ Pk.	0.40$^{\#}$ Pk.
0.77$^{\#}$ Pk.	0.60$^{\#}$ Pk.
0.82* Gr.	0.77$^{\$}$ Pk.
0.87$^{\#}$ Gr.	0.82* Gr.
0.97* Or	0.88$^{\#}$ Gr.
	0.97* Or.

*- intense, $^{\$}$- Moderately intense, $^{\#}$- Faint
Br.- Brown, Gn.- Green, Pk.- Pink, Gr.- Grey, Or.- Orange

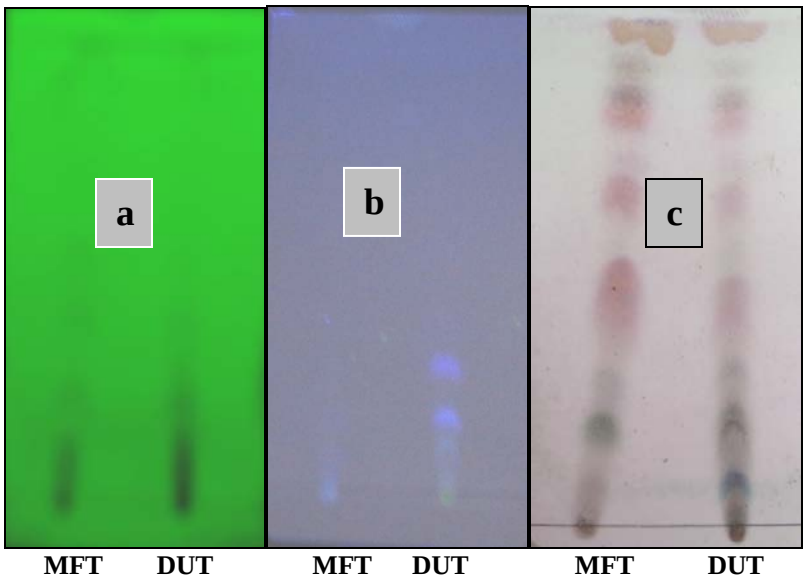

Figure3: TLC fingerprints of oil extract a) 254 nm, b) 366 nm and c) visible after spray in the Solvent system of Hexane: Dichloromethane: 1% Methanol in Dichloromethane (1: 4: 5 v/ v) mixture

The TLC of finger print of the dichloromethane extract of the oil sample showed twelve spots with R_f values ranging from 0.0167 to 0.9666 in the hexane: dichloromethane: 1% methanol in dichloromethane (1: 4: 5 v/ v) solvent system.

Table 5: TLC analysis of different brands of *Thalangai Ennai*

R_f values and colour of spots	
MFT	DUT
0.029* Br.	0.03* Br.
0.07* Gn.	0.06$ Pk.
0.14$ Gr.	0.07* Br.
0.20# Br.	0.11# Gr.
0.24* Pk.	0.19# Br.
0.28* Pk.	0.23$ Pk.
0.36# Pk.	0.26$Gr.
0.41* Pk.	0.36# Pk.
0.46$Gn.	0.40$Pk.
0.53# Pk.	0.44$ Gn.
0.59# Gn.	0.53# Pk.
0.73# Pk.	0.59# Gn.
0.79# Pk.	0.86# Pk.
0.90* Or.	0.91* Or.
0.96* Pk.	0.97* Pk.

*- intense, $- Moderately intense, #- Faint
Br.- Brown, Gn.- Green, Pk.- Pink, Gr.- Grey, Or.- Orange

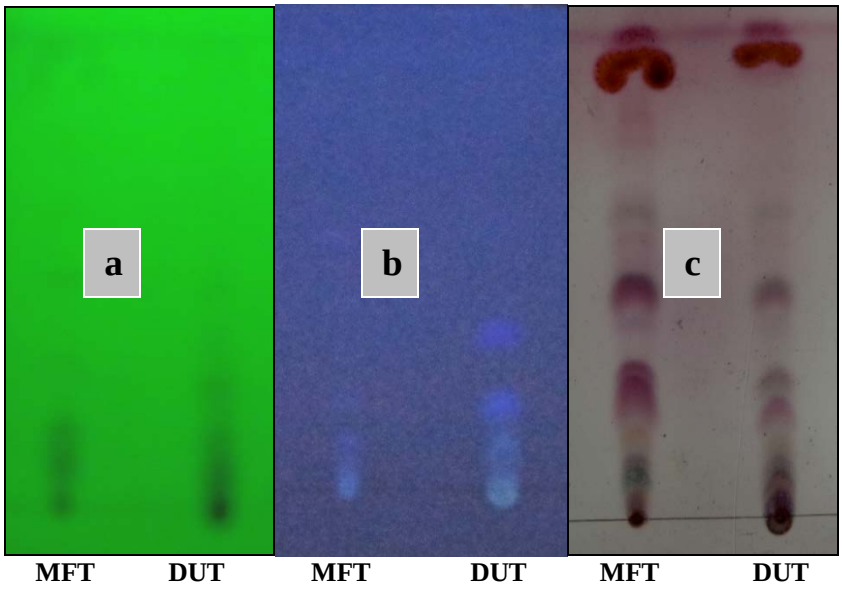

Figure4: TLC fingerprints of oil extract a) 254 nm, b) 366 nm and c) visible after spray in the Solvent system pure Dichloromethane

The TLC of finger print of the dichloromethane extract of the oil sample showed fifteen spots with Rf values ranging from 0.0286 to 0.9571 (MFT) and 0.0286 to 0.9714 (DUT) in the pure dichloromethane solvent system.

III. Discussion

The physicochemical standards available for the standardization of *Siddha* Medicated oils are insufficient. The analytical values available in the pharmacopoeia standards for *siddha* formulations are not finger print standards for each of the oil. The first step towards this goal, the current preliminary investigation was undertaken to generate data on physicochemical properties, including organoleptic characters, and chromatographic profiles to determine the quality and purity of T.E. It is very difficult to perform a study on Siddha medicated oils which has a large number of herbs used in the formulation. Hence in this study, only T.E was selected. As there is not any evidence for detailed physicochemical and TLC evaluation on T.E is reported. Therefore present work is taken up in the view to standardize the T.E. In the present study T.E was subjected to physicochemical parameters, organoleptic characters and TLC finger printing for preliminary standardization.

The results showed that, the specific gravity and pH value for T.EDUT were found to be 0.9185 and 4.61; in case of MFT these were found to be 0.9173 and 4.97 at room temperature (29^0C) respectively. The TLC finger print of both the formulations were comparable and revealed dichloromethane extract of the oil samples showed twelve spots with Rf values ranging from 0.0167 to 0.9666 in the hexane: dichloromethane: 1% methanol in dichloromethane (1: 4: 5 v/ v) solvent system and fifteen spots with Rf values ranging from 0.0286 to 0.9571 (MFT) and 0.0286 to 0.9714 (DUT) in the pure dichloromethane solvent system.

Although the separation of the oil extracts occurred in the solvent system comprised of hexane: dichloromethane: 1% methanol in dichloromethane (1:4:5 v/ v) as the mobile phase, the best separation of the oil extracts occurred in the solvent system comprised of pure dichloromethane (v) as the mobile phase. In dichloromethane extract of two brands of T.E **(**MFT and DUT) were analyzed by TLC .The number of spots with the Rf values were observed which were not remarkable differences in between these oils.

IV. Conclusion

The results obtained with the both brands of T.E **(**MFT and DUT) were found to be comparable and variations were not remarkable. These set of parameters presented in this paper can be used as reference standards for the quality assurance of T.E. Though the analytical values can be as preliminary reference standards, these values mostly related to the purity of the sesame oil, the qualitative and quantitative estimation of each single ingredient of T.E requires further research work in future.

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REFERENCES

[1] Kumar A and Baxi AJ, "Standardization of Ayurvedic Medicated Oil and the effect of Moorchhan on the amount of Marker in the oil", Indian Drugs, 44(2), February 2007, pp. 123-127.

[2] Thirunarayanan. T, "An Introduction to Siddha Medicine", Thirukumaran Publishers, Tiruchendur, 1988, 7-14.

[3] Kandaswami Muthaliyar, "Pathinnenn Siddher's Athmaratchamizthamenum Vaidthiya Sara sangiragam", Vata roga padalam, B Rathinanajakar & Sons.

[4] Kannusamipillai S, Chikitsa Rathina Theepam–Vaidhya chinthamani, Part II, B Rathinanajakar & Sons, Chennai, 1984; 6: 57-84.

[5] Ponniahpillai, I., "Vaithiya vihazham ennum Amirthasakara pathartha sudamanium vaidhya thelivum (Irupalai shettiyar)", Provincial Department of Indigenous Medicine, Ministry of Health, N.E.P., 2000: 35-36.

[6] Ponniahpillai I., "Pararajasekaram IV (Vatha roga nidhanam)", Committee for the Publication of Siddha Ayurvedic Medical Texts, Jaffna. 2001; 01-96.

[7] Kuppusamy Muthaliar KN; SiddhaMaruthuvam (Pothu). Indiamaruthuvam-Homeopathy thurai, Chennai 600106, 2004; 6: 20-21, 573-645

[8] Ponniahpillai I., "Sekarasasekara Vaidthiyam", Provincial Department of Indigenous Medicine, Ministry of Health, N.E.P. 2000b; 82-84, 106.

[9] Ramanathan P. Siddha Pharmacopoeia. Thanapalasaraswathy S.Municipal Council Jaffna, All Island Service Siddha Ayurvedic Medical Officers Union, Jaffna, 2000; 1: p. 108.

[10] Kirtikar KR, Basu BD, Indian Medicinal Plants, Bishen Singh Mahendra Pal Singh 2003, Vol. I – IV (pp. 84-729; 1422-1307; 2153-2640).

[11] Hepsibah PTA, Prasad NBR & Sanjeev Kumar P, "Standardization of Ayurvedic oils". Ancient Science of Life; 1998, 17(4): pp. 280 – 283.

[12] Murukesu Muthaliyar KS. Materia Medica I (Vegetable kingdom). Siddha maruthuva varrium, Chennai 600106, 1936; 1: 20-21, pp. 06-661.

[13] Thiyagarajan R, Gunapadam (Thathu Jeeva Vaguppu) part II & III, Directorate Indian Medicine & Homoeopathy, Chennai, IVth ed. 1992, 43-63; 2004; 4: 369-372, 401-405.

[14] Nadharni K, Indian Materia Medica, Vol. I, Vegetable kingdom, Bombay Popular Prakashan, 2002, pp. 64-934.

[15] Siddiqui, Hakim MA. "Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix), 24-25 January, New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995.

[16] http://www.engineeringcivil.com/determining-specific-gravity-of-bitumen.html June, 2012.

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