



**Research Article**

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## Standardization of *Thoravature Taila*: A Sri Lankan Traditional formulation used in Chronic Kidney Disease

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### ABSTRACT

**Background:** Ayurveda is a medicinal system which has a long history which goes back to 5000 B.C. Most of the time plant based formulations are used in Ayurveda system of medicine. Chronic kidney disease has been recognized as one of the non communicable diseases in Sri Lanka. *Thoravature Taila* is a traditional formulation which is mentioned under the Sri Lankan Ayurveda Pharmacopeia and is recommended specially for chronic kidney disease. In ancient time, herbal medicines were standardized based on the organoleptic characters. However, such an evaluation can be vary from individual to individual and is not adequate for industries in large scale. **Objective and Methods:** Therefore, an attempt was made to standardize *Thoravature Taila* in terms of physic-chemical parameters and development of Thin Layer Chromatography (TLC). **Results:** Specific gravity, refractive index, peroxide value, acid value, saponification value iodine value for *Thoravature Taila* were  $0.9580 \pm 0.0004$ ,  $1.3846 \pm 0.0012$ ,  $6.4 \pm 0.5$  milliequivalents/kg,  $0.4 \pm 0.0$  mg KOH/g,  $325.5 \pm 1.2$  mg KOH/g,  $16.2 \pm 0.4$  g I2100/g respectively. TLC fingerprint of *Thoravature Taila* was developed using ethyl acetate, methanol, cyclohexane and dichloromethane in a ratio of 0.2:0.2:1.6:3.0 v/v and observed under 254 nm and 366 nm. **Conclusion:** analytical data such as specific gravity, refractive index, peroxide value, acid value, saponification value and iodine value and TLC fingerprint were developed for *Thoravature Taila* for the first time and can be considered as the standard parameters when assessing the quality of this medicated oil.

**Keywords:** *Thoravature Taila*, Chronic kidney disease, Quality assessment.

### INTRODUCTION

Chronic kidney disease has been recognized as one of the non-communicable diseases in Sri Lanka. It is characterized by gradual loss of kidney function which persists for three or more months. Furthermore, in developing countries percentage of renal disease patients will be increased to 70% by 2030<sup>[1]</sup> due to economically nonviable treatment methods. Recent advances in biomedical research and novel technologies have created strategies to study kidney disease, diagnosis and treatment of individuals in variety platforms, applied to human populations. Up to today the treatment modalities designed by the expertise are unsuccessful to manage the Chronic kidney disease. However, since time immemorial conservative treatment has been used by the Sri Lankan Native and Ayurveda physicians showed significant improvement. *Thoravature Taila* is a Traditional formulation which is mentioned under the Sri Lankan Ayurveda Pharmacopeia<sup>[2]</sup> and is recommended specially for Chronic kidney diseases. It consists of leaves of *Clitoria ternatea* Linn, seeds of *Embelia ribes* Burm. f, Potassium Nitrate and Coconut oil (Fig. 1). In ancient time herbal medicines were standardized based on the organoleptic characters. However, such an evaluation can be vary from individual to individual and is not adequate to assess the quality of a drug. Furthermore, any research studies have not conducted to access the quality of *Thoravature Taila* yet. Therefore, an attempt was made to standardize the *Thoravature Taila* to confirm its identity, quality and purity for the assessment of the safety, efficacy and quality of the drug.

### MATERIALS AND METHODS

#### Collection of ingredients

Fresh leaves of *C. ternatea* and seeds of *E. ribes* were collected from Western Province of Sri Lanka during the period of November 2017 and authenticated by Senior Scientist, Bandaranayake Memorial Ayurveda Research Institute, Sri Lanka. Voucher specimens were deposited in Institute of Indigenous Medicine, University of Colombo, Sri Lanka. Potassium Nitrate and pure Coconut oil were purchased from the local market.

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Fig. 1: Ingredients of *Thoravature Taila*

#### Preparation of oil

*C. ternatea* (1 kg) fresh leaves were crushed well, added to water (4 L) and mixed well. Filtrate was poured to a stainless-steel vessel, 1L of virgin coconut oil was added and it was kept on the fire for heating. After 30 minutes, powder of *E. ribes* and Potassium Nitrate were added and stirred well. Then the contents were subjected to heating up to the stage of madyapaka and oil was extracted according to the procedure of oil preparation (Snehakalpana) as per the classical method. [3]

#### Organoleptic properties

Color, odor and appearance were observed for the *Thoravature Taila*.

#### Specific gravity

By using a specific gravity bottle at room temperature ( $25 \pm 2$  °C).

#### Refractive Index

By using a refractometer (ATAGO, Co, Ltd, Japan) at 25 °C.

#### Saponification value

Alcoholic potassium hydroxide (25 ml) was added into a conical flask containing *Thoravature Taila* (1.5g) and heated on the water bath for 1h. After 1 h, inner wall of the condenser was washed with 10 ml of ethyl alcohol. Phenolphthalein (1 ml) was used as the indicator. A blank titration was carried out at the same time. [4]

#### Peroxide value

A solution consists of acetic acid/chloroform was added into the glass stopper conical flask containing *Thoravature Taila* (5 g) and mixed well. After adding saturated potassium iodide (0.5 ml), allowed the solution to stand for one minute with occasional shaking. Then added 30 ml of distilled water and titrated with sodium thiosulphate solution (0.1 N) with constant and vigorous shaking. [5]

#### Acid value

Freshly neutralized hot ethyl alcohol (100 ml) and phenolphthalein (1 ml) were added to a conical flask containing 5 g of *Thoravature Taila*. The mixture was boiled for 5 minutes and titrated with standard 0.25 N KOH solution [5].

#### Iodine value

Both *Thoravature Taila* (5 g) and carbon tetrachloride (25 ml) were added into the iodine flask, and dissolved well. After that, Wijis solution (25 ml) was added and replaced the glass stopper after wetting with potassium iodine solution. Then allowed to stand in dark for 1 h. A blank test was carried out under similar experimental conditions. Potassium iodine solution (15 ml) and water (100 ml) were added, liberated iodine was titrated with standard sodium thiosulphate solution. [4]

#### Thin Layer Chromatography (TLC) fingerprint

*Thoravature Taila* (50 ml) and water (100 ml) were added to a round bottom, refluxed for 1 h, and water layer was separated. Water layer was added to a separating funnel containing dichloromethane (50 ml), mixed well and kept for 20 min. Finally, dichloromethane layer was separated. This was repeated for thrice and pooled dichloromethane fraction was concentrated and spotted on a TLC plate. As the mobile phase, a mixture of ethyl acetate, methanol, cyclohexane and dichloromethane in a ratio of 0.2:0.2:1.6:3.0 v/v was used and observed the TLC fingerprint under 254 nm and 366 nm.

#### Microbiological limits

*Thoravature Taila* was subjected to detect limits of aerobic plate count, Coliforms, and Yeast and Moulds according to the methods described in SLS standards. [6-8]

#### Heavy metal analysis

Quantitative determination of As [9], Hg [10], Cd [9] and Pb [9] were carried out for *Thoravature Taila*.

### RESULTS AND DISCUSSION

Quality assessment is very important for herbal preparations such as medicated oils, decoctions, infusions, pastes, powders, etc. in order to maintain the batch wise consistency. Therefore, many attempts have been carried out to establish the quality control parameters of herbal preparations in Sri Lanka. [11-14] In the present study, quality assessment study was carried out for *Thoravature Taila*, a traditional formulation used in chronic kidney disease. Specific gravity, refractive index, peroxide value, acid value, saponification value and iodine value of *Thoravature Taila* is given in Table 1.

Table 1: Physicochemical parameters of *Thoravature Taila*

| Parameters                  | <i>Thoravature Taila</i>         |
|-----------------------------|----------------------------------|
| Specific Gravity (at 25 °C) | 0.9580 ± 0.0004                  |
| Refractive Index (at 25 °C) | 1.3846± 0.0012                   |
| Saponification Value        | 325.5±1.2 mg KOH/g               |
| Peroxide Value              | 6.4±0.5 milliequivalents/kg      |
| Acid Value                  | 0.4±0.0 mg KOH/g                 |
| Iodine Value                | 16.2± 0.4 g I <sub>2</sub> 100/g |

Values are expressed as Mean ±SEM, n = 6

Organoleptic characteristics such as color, smell and appearance are the preliminary identification of *Thoravature Taila*. It was greenish black in colour, viscous in appearance, aromatic in odor and pungent in taste. Similar research work have been carried out for medicated oils such as *Vipadikahara Grita Taila* [11], *Mustadi Taila* [12], *Yashtimadhukadi Taila* [15-16] and *Bhrngamalkdi Taila*. [17] Specific gravity and refractive index of *Thoravature Taila* were comparable to that of coconut oil which used as the base oil for the preparation of this medicated oil. Iodine value is often used to determine the amount of unsaturated fatty acids and the lower iodine value of *Thoravature Taila* indicates the stability of the oil as well as resistance for oxidation and free radical production. [18, 19] Saponification value of the oils originated from plants should be in a range of 188 to 196 mg KOH/g oil. [20] In contrast, saponification value of *Thoravature Taila* was beyond the given range. However, similar saponification values were observed for some other medicated oils such as *Karpooradi Taila* [21], *Yashtimadhukadi Taila* [16] and *Lashuna Taila*. [22] TLC is a one of the convenient, cheap and simple technique to detect the phytochemical profiles of herbal drugs. In the present study, TLC fingerprint profiles were developed for *Thoravature Tailaya* at 254 nm and 366 nm using ethyl acetate, methanol, cyclohexane and dichloromethane in a ratio of 0.2:0.2:1.6:3.0 v/v as the mobile phase (Fig. 2).

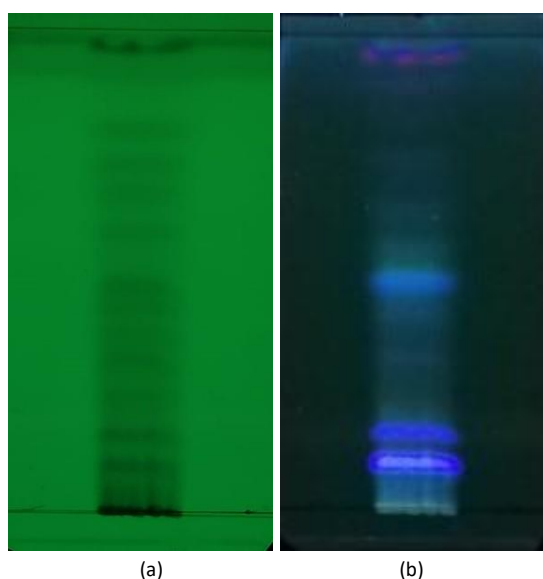


Fig 2: TLC fingerprint profile of Thoravature Taila at (a) 254 nm and (b)366 nm

Pathogenic microorganisms such as aerobic bacteria, Coliforms, Yeast and Moulds were not found in *Thoravature Tailaya*. In addition, toxic heavy metals such as As, Hg, Cd and Pb were well below in the *Thoravature Tailaya* compared to the recommended upper limits for heavy metals in Canada (As:5 ppm, Hg:0.2 ppm, Cd:0.3 ppm and Pb:10 ppm) for herbal medicines.

## CONCLUSION

Standardization of *Thoravature Tailaya* was carried out for the first time and output of the research can be considered as the standard parameters when assessing the quality of this medicated oil.

## Conflict of interest

None

## Source of Support

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