

J Ayu Med Sci -

Quarterly Journal for Rapid Publication of Researches in Ayurveda and Other Traditional Medicines

# Journal of Ayurveda Medical Sciences



J Ayu Med Sci | 2017 | Vol 2 | Issue 3 (Jul – Sep)

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## Analytical Standards of *Vrukshamla* (*Garcinia indica* Choisy) *Beeja Taila* - Kokum Butter

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## ABSTRACT

Introduction: Garcinia indica Choisy is the most commonly accepted source of Vrukshamla, which is one of the commonest herbs mentioned in classics of Ayurveda. G. indica is also known as kokum butter tree as its seeds yield an oil, which remains solid at room temperature. This extracted oil from the seeds of Vrukshamla is used by folklore Vaidyas in Western Ghats of India to treat various skin ailments like dry skin conditions, cracked heels etc. Methods: The present study has been undertaken to evaluate and analyze the Vrukshamla beeja taila (VBT) by standard oil parameters, including HPTLC. Results: The sample of VBT was solid at room temperature, having yellowish/offwhite colour and characteristic odour. Its analytical study showed the presence of fatty acids as esters, which were fresh not rancid. TLC photo documentation revealed presence of many phyto-constituents with different Rf values. HPTLC densitometric scan of the plates showed numerous bands under 254 nm, 366 nm and 620 nm (after derivatisation). On documentation, there were 3 spots under 254 nm, 4 spots under 366 nm and 12 spots under 620 nm postderivatisation with vanillin sulphuric acid spray reagent. Conclusion: This study will serve as a standard reference for preliminary identification of VBT.

## KEYWORDS

Kokum butter, Oil analysis, Vrukshamla.

## PICTORAL ABSTRACT Kernel-Powder (1-part) Adding water (3-parts) Physico-chemical Analysis of Kokum butter by Standard oil parameters After cooling: Mould of Kokum butter HPTLC

## ARTICLE HISTORY

Received 29.07.2017 Accepted 31.07.2017

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## CITE THIS RESEARCH AS

Mohammed F, Joshi SV, Patgiri B, Bairy ST, Narayana SK. Analytical Standards of *Vrukshamla* (*Garcinia indica* Choisy) *Beeja Taila* - Kokum Butter. J Ayu Med Sci 2017;2(3):247-50.

DOI 10.5530/jams.2017.2.21

Garcinia indica Choisy (Figure 1), syn. G. purpurea Roxb. And Brindonia indica Dupetit-Th. (Family Clusiaceae) is the most commonly accepted source of Vrukshamla, one of the commonest discussed drugs in the classics of Ayurveda. The species was firstly described by Dr. Garcia in 1574, hence the genus name is Garcinia and as it's endemic to India, the species name is indica. The word purpurea is originated from a latin word Purpura, means purple colour; as the colour of its ripe fruit. [1-3] It's a small, slender, fruitbearing tree, having very strong and sturdy growth; leaves are approximately 10 cm × 5 cm in dimensions; having red-tinge, when

young, which turns in dark green on maturation; oblong, ovate or elliptic-lanceolate, rarely obtuse; glabrous, membranous, mucronate; with rounded, acute or abruptly acuminate apex and narrowed it base. Petiole is up to 0.7 cm long. Flowers are polygamous. Male flowers are 3 to 8 in number, axillary and/or terminal fascicles, solitary or in a spreading fascicles; with 4-sepals, decussate, outer ones smaller than inner sepals; whereas female flowers are sessile/sub-sessile, solitary or 2 to 3 together, terminal, shortly peduncled. [4,5] Flowering season is November to February. Fruits, commonly known as kokum fruits, are globose and resemble a small apple in size, not furrowed/grooved. [4]

## Figure 1. Garcinia indica Choicy







It's green, when unripe and turns dark red/purple on maturation, which usually happens by April/May in each year. Fruit-pulp is dark in colour and little-sweet in taste. Each fruit contains 5 to 8 big, flat seeds embedded in juicy pulp.<sup>[1]</sup> The seed coat is fibrous from outside; each seed is dicotyledonous, reniform-shaped and has a dimension of about 1.875 cm  $\times$  1 cm, which contains 23% to 26% (c. 44% on the weight of kernels) oil. [6] Extracted seed-oil, known as kokum butter, remains solid at room temperature.[1] It's sold in market in form of oval-shaped moulds/lumps or cubical cakes. Its colour is grayish/yellowish white and granular, greasy consistency. [6] There are two methods of extraction of kokum butter from the seeds, viz. the traditional boiling method and the churning method.<sup>[7]</sup> Kokum butter is a specific remedy in dysentery and mucous diarrhea, administered in doses of 1 tola in a quarter seer of milk thrice a day until complete recovery; it's useful in Phthisis pulmonalis and some scorbutic (skin) diseases. It has been recommended as a substitute for cod-liver oil, and is eaten by poor people as a substitute for ghee. Externally, oil has a healing property and might be usefully employed as an application to ulcerations, fissures of lips, hands, chapped skin, etc. [8]

According to classical Ayurvedic texts, its unripe fruit is *Amla* (sour), whereas ripe fruit is *Katu* (hot and spicy), *Kashaya* (Pungent)/*Amla* (Sour), *Alpa madhura* (Little sweet).<sup>[3]</sup> It's *Guru*, *Ruksha* and *Sangrahee*.<sup>[9]</sup> It's *Veerya* is *Ushna* and *Vipaka* is *Amla*. Its bark and oil is *Vranaropaka* (accelerates the wound healing) and *Stambhanakara*.<sup>[10]</sup> Its fruit rind possesses weight reduction properties due to 3-hydroxy citric acid.<sup>[9]</sup> It's also semen coagulant and CNS depressant.<sup>[9]</sup>

In this study the kokum butter was prepared by traditional boiling method and analyzed for standard oil parameters.

## Plant Material

Seeds of *Vrukshamla* (*Garcinia indica* Choisy) were collected from Udupi district and surrounding forest area of South Kanara.

## Preparation of VBT

Traditional boiling-method was followed in this study; for which the collected seeds were dried completely under sunlight. Thereafter, the kernels were obtained by removing the seed-coat manually. Kernels were further subjected to trituration with sufficient quantity of water, for the preparation of paste. That was mixed with 3 litres of water (per kg of seed-kernel) in a vessel and subjected to heat on mild flame for very long time. After some time, a layer of oil started floating on the water, which was skimmed off and taken into another vessel. This second vessel was also simultaneously subjected to the heat on the other stove, until the remnant of water evaporates and the oil remains alone. This oil was allowed to cool down at room temperature and solid Kokum butter was obtained. Heating the first vessel was continued until no

or negligible quantity of oil floated. Obtained quantity of Kokum butter was 40 gm per kg of seed-kernel.

## **Analytical Study**

Following physic-chemical parameters of VBT were studied, as per the testing protocol of ASU medicines. Specific gravity, Rancidity test (Kreis Test), Refractive index, Determination of Acid value, Determination of Saponification value, Determination of unsaponifiable matter, Iodine value, Ester value, Colour, Odour, Viscosity, Determination of peroxide value and Chromatographic analysis (HPTLC). [11]

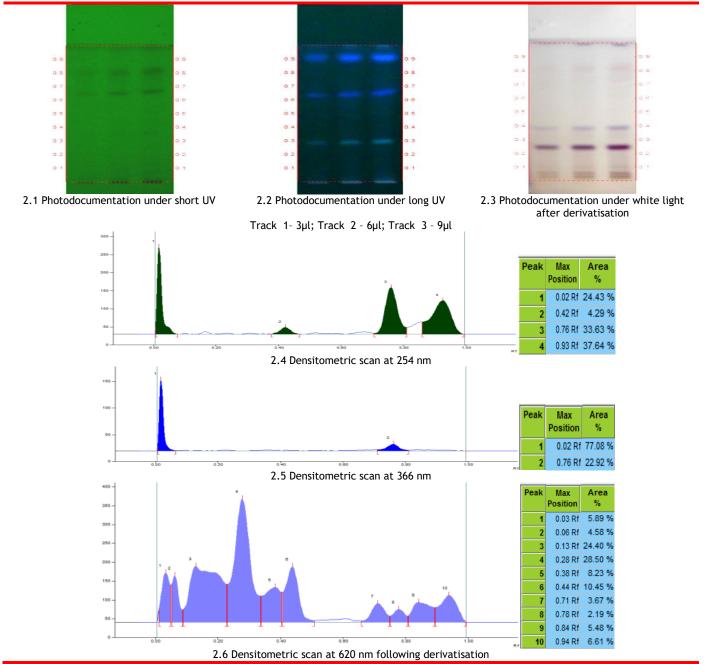
The specific gravity of VBT was found to be 0.9478. It was not oxidized when rancidity rest (Kreis Test) was performed. Its refractive index was found to be 1.46944. Its determined Acid value and Saponification value were 75.69 and 165.49 respectively; whereas, the unsaponifiable matter was found to be 0.59%. Its calculated lodine value and Ester value were 24.40 and 89.80 respectively. Colour was Yellowish/off-white and the odour was characteristic. The viscosity test could not be performed due to the very thick consistency of the sample, as it could not pass through the tube of viscometer. The calculated peroxide value of VBT was found to be 0.85 (Table 1).

TLC photo documentation revealed presence of many phytoconstituents with different  $R_f$  values and HPTLC densitometric scan of the plates showed numerous bands under 254 nm, 366 nm and 620 nm (after derivatisation). On Photo documentation, there were 3 spots under 254 nm, 4 spots under 366 nm and 12 spots under 620 nm post-derivatisation with vanillin sulphuric acid spray reagent. Densitometric scan at 254 nm revealed 4 peaks corresponding to 4 different compounds in the chloroform extract, compounds with  $R_f$  0.02 (24.43%), 0.42 (4.29%), 0.76 (33.63%) and 0.93 (37.64%) were 4 peaks. The densitometric scan at 366 nm showed 2 peaks; the first with  $R_f$  0.02 (77.08%) and the second with  $R_f$  0.76 (22.92%). Then, at 620 nm following derivatisation, the densitometric scan showed 10 peaks corresponding to 10 different compounds, compounds with  $R_f$  0.28 (28.50%), 0.13 (24.40%), 0.44 (10.45%), 0.94 (6.61%) and 0.03 (5.89%) were the major peaks (Table 2 and Figure 2).

Table 1. Standard oil parameter results of *Vrukshamla beeja* taila (Kokum butter)

tura (Rokum Butter)		
Parameter	Result	
Specific Gravity	0.9478	
Rancidity	Not oxidized	
Refractive Index	1.46944	
Acid value	75.69	
Saponification value	165.49	
Unsaponifiable matter	0.59 %	
Iodine value	24.40	
Ester value	89.80	
Colour	Off-white/Yellowish white	
Odour	Characteristic odour	
Peroxide value	0.85	

## Figure 2. HPTLC of chloroform extract of Vrukshamla beeja taila (Kokum butter)



Solvent system: Toluene: Ethyl acetate (8:0.5)

Table 2. Rf values of Chloroform extract of Vrukshamla beeja taila (Kokum butter)

At 254 nm	At 366 nm	After derivatisation
-	<u>-</u>	0.07 (Dark purple)
-	<u>-</u>	0.10 (Light purple)
-	<u>-</u>	0.15 (Light purple)
-	<u>-</u>	0.18 (Light purple)
-	<u>-</u>	0.25 (Dark purple)
-	0.30 (Fluorescence-aqua blue)	-
-	-	0.34 (Light purple)
0.36 (Light green)	-	-
-	-	0.39 (Dark purple)
-	0.50 (Fluorescence blue)	-
-	-	0.52 (Light purple)
0.65 (Dark green)	0.65 (Fluorescence-Dark blue)	0.65 (Light purple)
-	<u>-</u>	0.68 (Light purple)
<del>-</del>	-	0.72 (Light purple)
0.82 (Dark green)	<u>-</u>	0.82 (Light purple)
<del>-</del>	0.91 (Fluorescence-Dark blue)	<u>-</u>

This documented analytical study of VBT can be used to judge the adulteration and purity of it, as it was performed by standard oil parameters. HPTLC fingerprinting will help to supplement information in regard to its identification and standardization.

**ACKNOWLEDGEMENTS** The authors are thankful to SDM Centre for Research in Ayurveda and Allied Sciences, Udupi for providing essential facility and support for the study.

## SOURCE OF SUPPORT Nil

## CONFLICT OF INTEREST None declared

**Contributors** Dr Sreekant contributed to design, literature study and data acquisition and analysis. Dr Faisal and Dr. TS Bairy contributed to the conceptualization of the topic, manuscript review and analysis. Dr. Sunil guided in analysis and manuscript preparation.

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