



Research Article

Assessment of Quality Parameters and Identification of Garcinol in Vrikshamla (*Garcinia indica*) Using HPTLC- An Analytical Study

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Abstract

The tropical evergreen tree *Vrikshamla* (*Garcinia indica* Linn.), commonly referred to as *kokum*, has many health advantages, such as anti-inflammatory, anti-obesity, and antioxidant qualities. It contains bioactive substances with therapeutic uses for diseases like Dyslipidemia, cancer, diabetes, and cardiovascular disorders, such as anthocyanins, hydroxycitric acid, and garcinol. For *Vrikshamla* capsules to be consistent and effective, quality control and validation are crucial. Material and Methods: *Vrikshamla* capsules were procured from SDM Ayurveda Pharmacy in Udupi. Organoleptic, physicochemical (water-soluble extract, alcohol-soluble extract, pH, loss on drying, and heavy metals), microbiological characteristics and High Performance Thin-Layer Chromatography (HPTLC) were the main focus of the quality analysis, which was carried out in the Central Research Facility in accordance with in-house standard values requirements. Results: High water-soluble (94.71%) and alcohol-soluble (87.77%) extracts with a pH of 3.89 that fell within the normal range were shown by the physicochemical examination. After drying, the heavy metals were in compliance (≤ 20 ppm), and the loss was 1.12%. The absence of pathogens such as *Salmonella* spp., *P. aeruginosa*, and *E. coli* was verified by microbiological testing. Garcinol's existence in the sample was confirmed by HPTLC analysis, which detected it at R_f 0.63. Discussion: According to the findings, *Vrikshamla* capsules are of a high calibre, and their pharmacological qualities are attributed to active ingredients like garcinol. Its application in the treatment of metabolic and chronic disorders is supported by its anti-inflammatory, anti-obesity, and neuroprotective properties. Conclusion: *Vrikshamla* capsules therapeutic value, especially for dyslipidemia, obesity, and inflammation, is confirmed by quality control and validation, underscoring its significance in Ayurvedic medicine.

Keywords: *Vrikshamla*, *Garcinia Indica*, Garcinol, Quality Control, Ayurvedic Medicine, HPTLC, Bioactive Compounds, Anti-Inflammatory, Anti-Obesity, Pharmacological Properties

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Introduction

Vrikshamla (*Garcinia indica* Linn.), commonly known as *kokum*, is a tropical evergreen tree that belongs to the Clusiaceae family, which is related to the mangosteen family. It is found in western ghats of India. (1) Among its attributes are *Amla rasa* (sour taste)

in unripe fruit, *Madhura amla rasa* (sweet and Sour taste) in ripe fruit, *Laghu-ruksha* (light and dry) in *guna* (Qualities), *ushna virya* (hot potency), *amla vipaka* (post-digestive effect of sour taste), *Kapha-vatashamak* (pacifying both Kapha and Vata doshas), and *hridya* in *prabhav* (Influence on Heart). (2) The health benefits of *kokum* have been associated with a variety of bioactive compounds, such as phenolic acids, flavonoids, and citric acids. Among these, garcinol hydroxycitric acid and anthocyanins (cyanidin-3-glucoside and cyanidin-3-sambubioside) are the primary bioactive ingredients. Numerous medicinal applications for a variety of conditions, such as dyslipidaemia, cancer, inflammation, diabetes, obesity, cardiovascular disease, and neurological issues. It has also been shown to have antibacterial, anti-inflammatory, anti-obesity, anti-arthritis, and antioxidant properties in *G. indica* fruit and fruit rinds. (3) In

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today's era most of the apothecaries by recognizing its properties, started to prepare this drug in the form of different dosage forms like Capsules or in the form of *Kashayas*. It is crucial to assess *Vrikshamla*'s quality control parameters and authenticate them in accordance with accepted standards in order to conserve its quality in all forms and deliver the utmost quality of product.

Aims and objectives

- Aim of the study is to assess the quality standards of *Vrikshamla* (*Garcinia indica* Linn.).
- Objective of the study is to analyse the quality of the *Vrikshamla* (*Garcinia indica* Linn.) as per the inhouse specifications and quantification by instrumental analysis to know the active ingredients.

Materials and Methods

Capsule *Vrikshamla* was procured from SDM ayurveda pharmacy, Udupi, Batch No: 10GI2206. In this study, the Central Research Facility of SDM ayurveda pharmacy, Udupi conducted the quality analysis and validation of *Vrikshamla* in the form of churna, which was packed in capsule form. The analytical parameters that were assessed are organoleptic, physicochemical parameters (water soluble extract, alcohol soluble extract, pH 1% w/v solution, loss on drying, heavy metals) like and microbiological which were as per the in-house standard values which is set by repeated analysis of *Vrikshamla*.

Results for capsule Vrikshamla (Extract)

Physiochemical Test (Table No. 1)

The pH (4), water and alcohol-soluble extract (5), loss on drying (6)(7) and heavy metals (8) were assessed according to the guidelines mentioned in API and these results were as per the in-house standard values which is set by repeated analysis of *Vrikshamla*.

Table 1: Physiochemical Test

Sr. No.	Specified Physiochemical test	Results	Specification
1	Organoleptic Description	Brown Coloured dry powder with characteristic odour and taste	Brown Coloured dry powder with characteristic odour and taste
2	Water soluble extract	94.71%	Not less than (NLT) 70%
3	Alcohol soluble extract	87.77%	Not less than (NLT) 70%
4	pH 1% w/v solution	3.89	3-7
5	Loss on Drying	1.12%	Not more than (NMT) 7%
6	Heavy metals	Complies	Not more than (NMT) 20 ppm

Microbiological screening

All the test done in microbiology part were assessed according to the guidelines mentioned in API and these results were as per the in-house standard values which is set by repeated analysis of *Vrikshamla*. (9) Preparations of samples for Microbial Limit Test (MLT) and Total Microbial Load (TML). These Samples were buffered using Soyabean Casein Digest Medium (SCDM).

Following this step, inoculation of samples for both MLT and TML was performed. The sample were then inoculated on Soybean Casein Digest Agar (SCDA) and Sabouraud Dextrose Agar (SDA) media to determine Total Bacterial Count (TBC) and Total Fungal Count (TFC), respectively. Also, in Addition the samples were inoculated on Cetrimide Agar (CA), MacConkey Agar (MA), Mannitol Salt Agar (MSA), Xylose Lysine Deoxycholate Agar (XLD) media for the identification of obnoxious bacteria. The inoculated samples were incubated at 37°C for bacterial growth and at room temperature for fungal growth. (9)

The results were read to identify the presence of specific micro-organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella species*, TBC and TFC using the respective media MSA, CA, MA, XLD, SCDA and SDA. (9)

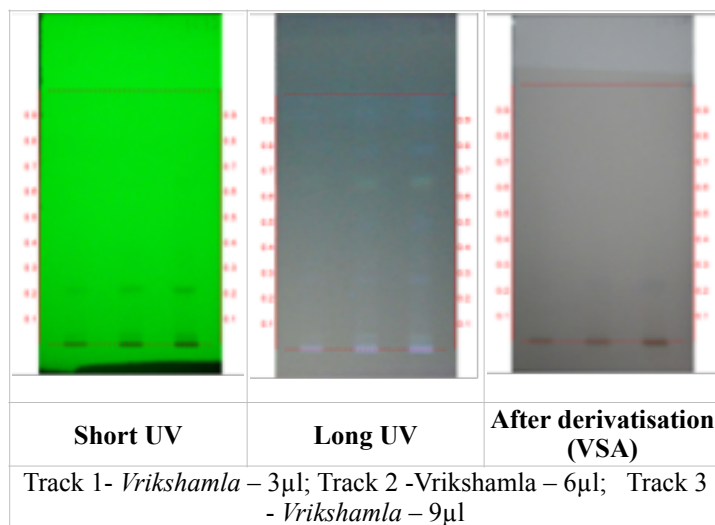
Table 2: Microbiological Screening

Sr. No.	Microbiological test	Results	Specification
1	Total plate count	Complies	Not more than (NMT) 1000 cfu/gm
2	Total yeast count	Complies	Not more than (NMT) 100 cfu/gm
3	E. coli	Absent	Absent
4	Salmonella Spp.	Absent	Absent
5	S. aureus	Absent	Absent
6	P. aeruginosa	Absent	Absent

HPTLC (High-performance thin layer chromatography)(10)

1 gm of sample of *Vrikshamla* was dissolved in 10.0 ml of ethanol and kept for maceration at room temperature for 24 hrs followed by filtration. 3µl, 6µl and 9µl each were applied on a pre-coated silica gel F²⁵⁴ on aluminium plates to a band width of 7mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid (4.0:1.0:0.5) in a double trough chamber. The developed plates were visualized in short UV, long UV and derivatised with Vanillin sulphuric acid reagent (VSA) and observed. Scanning was done at 254nm for the presence of Garcinol, R_f, colour of the spots and densitometric scan were recorded.

Figure 1: HPTLC photo documentation of Ethanol extract of Vrikshamla

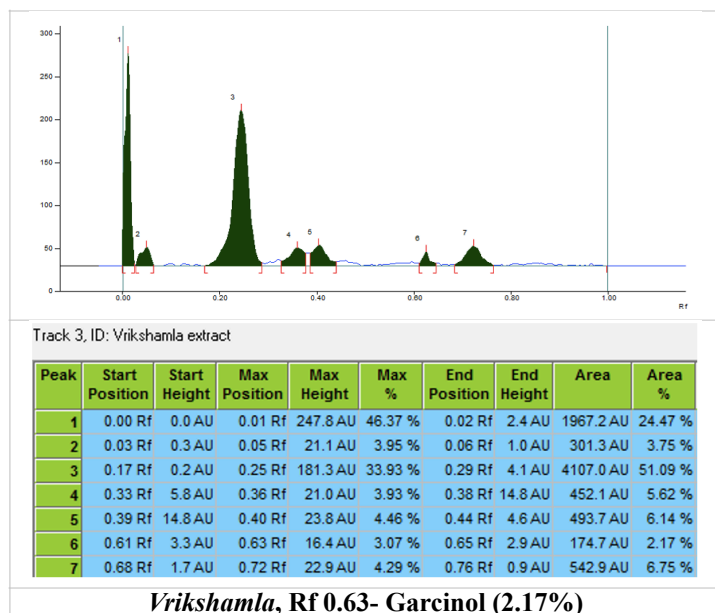


Solvent system –
Toluene: Ethyl acetate: Formic acid (4.0:1.0:0.5)
Volume of Solvent Added (VSA), 254nm
Fingerprint 0.31, 0.34, 0.37 0.47, 0.54, 0.63 (Garcinol), 0.83, 0.93

Table 3: Rf value of *Vrikshamla*

Sr.	Short UV	Long UV	After
1	---	0.05 (F. blue)	---
2	0.22 (Green)	---	---
3	---	0.27 (F. blue)	---
4	---	0.66 (F. green)	---
5	---	0.79 (F. blue)	---
6	---	0.93 (F. blue)	---

Figure 2: Densitometric scan of *Vrikshamla* at 254nm



Garcinol at Rf 0.63 is witnessed in *Vrikshamla* and it is important to determine it because it is the active compound present in *Garcinia indica* by which it is proved that the capsule prepared from the churna is actually a genuine drug. The actual quantity of Garcinol present in *Vrikshamla* is not Standardized yet but various article shows the reference range of Garcinol nearly to the percentage found in the HPTLC analysis. (11)(12)

Discussion

Vrikshamla is one of the most important herbal remedies in Ayurveda. Here, the *Vrikshamla* has been analysed having brown coloured dry powder with characteristic odour and taste. The physiochemical test was found to be within the ranges specified, water and alcohol soluble extracts were 94.71 and 87.77 % respectively, which are more than 70%. The pH 1%w/v solution 3.89 within the normal range mentioned as per the in-house standards (3-7), loss on drying is 1.12% which is less than the standard values mentioned which means moisture content is less and the product has long shelf life which helps in the long preservation and storage for longer period of time and when tested for the heavy metals it complies within normal ranges (Table No. 1) and under microbiological test all the parameters such as total plate count and total yeast count were complies within normal range and for *E. coli*, *Salmonella spp.*, *S. aureus*, *P. aeruginosa* all were absent as per the specification mentioned in-house standard values (Table No.2) and along with that HPTLC is also done with

densitometric scan at 254nm which shows the presence of Garcinol at Rf value of 0.63. (Figure 2).

According to various chemical studies, the rind contains poly-isoprenylated phenolics (garcinol and iso-garcinol), anthocyanins (cyanidin-3-glucoside, cyanidin-3-sambubioside), carbohydrate, tannin, pectin, as well as organic acids such as (–)-hydroxycitric acid, and citric acid.(13)(14) In the physiochemical test, a higher water-soluble extract for *Vrikshamla* (*Garcinia indica*) indicates that a sizable amount of water-soluble chemicals is present in the plant. (15) Important secondary metabolites such polyphenols, flavonoids, and organic acids which helps in reducing oxidative stress and inflammation, antioxidants and helps in weight management. (16)(17) Higher concentrations of alcohol-soluble chemicals indicate the existence of potentially advantageous bioactive molecules such as terpenoids, sterols, triterpenes, essential oils, and flavonoids with anti-oxidant, anti-inflammatory, antibacterial, immune-boosting, and analgesic properties. (18)(19) pH is acidic in nature due to the presence of hydroxy citric acid which further helps in metabolism of fat and helps in weight management. (20)(15) On the other hand, Garcinol the most active compound found using HPTLC is a poly-iso-prenylated phenol which is of higher therapeutic uses having anti-dyslipidaemic by regulating lipid synthesis and fatty acid oxidation, anti-obesity, anti-oxidant due to which it neutralizes free radicals and helps in chronic and degenerative conditions, anti-inflammatory due to which it helps in conditions like arthritis, inflammatory bowel diseases, anti-cancerous by inducing apoptosis and cell cycle arrest, inhibiting of angiogenesis, neuroprotective by functioning as a histone acetyltransferase inhibitor, anti-ulcer, anti-HIV, anti-bacterial, Hepatoprotective, anti-depressant, cardioprotective properties. (1)(21) While the other active compounds like anthocyanins is also a potent anti-oxidant, having anti-inflammatory properties, enhance cognitive functions, helps in reducing insulin resistance and also helps in weight management by promoting fat burning. (22) The next active compounds are hydroxy-citric acid which supports weight loss (by reducing fat synthesis and using stored fat), appetite suppressor (by increasing level of serotonin which reduced cravings), mood enhancer by increasing serotonin level, helps in managing dyslipidemia. (23) Carbohydrates present in *Vrikshamla* have various therapeutic properties such as weight management, blood sugar regulation, digestive health support, antioxidant and anti-inflammatory effects. (24) Tannins present in *Vrikshamla* also have antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. (24) (25)

Conclusion

The quality control parameters and validation of capsule *Vrikshamla* extract churna found to be in the standard norms. The presence of Garcinol, in the concentration of 2.17% which supports the therapeutic efficacy of the Garcinol in the management of various disease such as dyslipidemia, arthritis, Inflammatory bowel diseases, HIV, ulcer, type 2 DM.

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Conflict of interest

There is no conflict of interest.

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