

PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF *VARUNA TAILA* THROUGH HPTLC (HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY)

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ABSTRACT

Background- *Varuna taila*, is an herbal ayurvedic medicine mentioned in *Bhaishjya ratnavali* and *Adarsha nighantu*, includes the leaves of *Createva nurvala* (*Varuna*), processed with *Createva nurvala* (*Varuna*) juice (*Swarasa*) & *Tila Taila* and used as a remedy for urinary tract infections (UTIs), Striae Gravidarum. This formulation is prepared to be given in Striae gravidarum (*Kikkisa*) in Pregnancy. There are several methods available today for managing stretch marks, which could be expensive and painful. Ever more important is the knowledge imparted to a worried mother that her child will be fine and that the management will be without complication and low price. Therefore, there is a need to develop standardized Ayurvedic formulation in new dosage form that is safe and effective for pregnant women.

Aim- The primary objective of this research was to conduct physicochemical standardization and high performance thin layer chromatography (HPTLC) analysis of *Varuna Taila*.

Results- The Pharmaceutical assessment of the oil revealed that it exhibited a yellowish green color. Loss on Drying is 0.09, Total Ash value is 0.596, pH is of 4.3, Refractive index is 1.4500, Specific gravity is 1.0901, Viscosity is 35.2960, Acid value is 0.8976, Rancidity test is Negative, Saponification value is 210 & Iodine value is 97. HPTLC analysis of *Varuna Taila* was performed using the CAMAG Linomat 5 HPTLC system. The outcomes were presented as chromatograms, with peaks depicted after scanning at wavelengths of 254 nm, 366 nm, and 540 nm. The drug's phytochemical profile was assessed and illustrated through tables that displayed various parameters including the total count of peaks, peak heights, peak areas, percentage area, and Rf values.

Conclusion- The present study focused on the phytochemical characterization of *Varuna taila*. The HPTLC profile generated can serve as a preliminary tool for ascertaining the authenticity and standardization of the formulation. While *Varuna taila* has been traditionally indicated for conditions such as striae gravidarum.

INTRODUCTION

High-Performance Thin Layer Chromatography (HPTLC) is a powerful analytical technique used for the qualitative and quantitative analysis of complex mixtures. This method is highly valuable in various fields such as pharmaceuticals, food, and environmental analysis due to its sensitivity, resolution, and versatility.

The preparation of the test solution is a critical step in ensuring accurate and reproducible HPTLC results. In this procedure, approximately 10 grams of the sample is initially weighed and placed in an evaporating dish. The sample is then subjected to evaporation until dryness or until a residue is obtained. Following this, 1 to 2 mL of methanol is added to the residue, and the solution is filtered using a syringe filter to obtain a clear test solution suitable for HPTLC fingerprinting.

The analytical process involves using a specific spray reagent for visualization and detection. For this purpose, an anisaldehyde-sulphuric acid reagent is prepared by mixing 0.5 mL of anisaldehyde with 10 mL of glacial acetic acid, 85 mL of methanol, and 5 mL of concentrated sulphuric acid. This reagent is used to enhance the visibility of compounds on the chromatographic plate.

The chromatographic conditions are carefully controlled to achieve optimal separation of the components. The sample is applied using a CAMAG Linomat 5 applicator with a start position of 10 mm and an end position of 80 mm from the plate base. The sample application volume is set to 4 µL with no distance between tracks. The development of the chromatogram is carried out in a CAMAG TLC Twin Trough Chamber, which is saturated for 30 minutes with a mobile phase consisting of toluene, ethyl acetate, and formic acid in a 7:3:0.1 v/v ratio.

Visualization of the chromatogram is performed at different wavelengths: 254 nm, 366 nm, and 540 nm after derivatization with the anisaldehyde-sulphuric acid reagent. The derivatization process is conducted using a CAMAG dip tank for approximately 1 minute, followed by drying of the plate in a TLC plate heater preheated to $100 \pm 5^\circ\text{C}$ for 3 minutes.

This systematic approach ensures the precise identification and quantification of the components present in the sample, allowing for effective quality control and analysis in various applications. Ayurveda, the ancient science of life, emphasizes both preventive and curative measures for maintaining health and managing diseases. Antenatal care (*Garbhini Paricharya*) is one such essential aspect that ensures the well-being of both the expectant mother and the growing fetus^[1].

Pregnancy is a transformative phase in a woman's life, and the primary objective of antenatal care is to safeguard the health of both mother and child. However, it is equally important to recognize the psychological impact that certain physical changes—such as stretch marks (striae gravidarum)—may have on a pregnant

woman during this period. The etiopathogenesis involves a combination of genetic factors, hormonal factors, and increased mechanical stress on connective tissue.^[2]

Striae gravidarum slightly depressed linear marks with varying length and breadth found in pregnancy. They are predominantly found in the abdominal wall below the umbilicus and sometimes over the thighs and breasts due to weakening of elastic tissues, associated with pregnancy. These are also known as striae atrophic and are commonly known as Stretch Marks. It causes much psychological distress to the women. It is a very major problem in the modern cosmetic conscious era.^[3] Striae Gravidarum may compound this stress for some women, negatively affecting their psychological and emotional wellness.^[4]

For the prevention of striae gravidarum in ancient Samhita like Bhaishjya Ratnavali & Adarsha Nighantu^{[5][6]} has mentioned *Varuna Taila* which is useful in treatment of striae gravidarum.

MATERIAL AND METHOD

The raw drug *Crateva nurvala* (*Varuna*) was collected from its natural habitat and got authenticated by department of *Dravyaguna* at Parul Institute of Ayurved, Parul University. A voucher specimen was deposited for future reference.

The leaves of *Crateva nurvala* (*Varuna*) were taken. The preparation of a standard sample of *Varuna Taila* was as per the *Sharangdhar Samhita*. The samples were prepared under the supervision of GMP-approved well established Parul Ayurved Pharmacy.

Drug review:

Crateva nurvala (*Varuna*) has a Bitter (*Tikta*), Astringent (*Kashaya*) taste, is Light (*Laghu*) and Dry (*Ruksha*) in properties, with *Ushna* (hot) potency, and *Katu* (pungent) post-digestive effect. It balances *Kapha-Vata*^[7] ^[8] doshas and contains L-Stachydrine, Dodecanac anhydride, Methyl pentacosanoate, Kaemferol-o- α -D glucoside, Quercetin-3-O- α -D glucoside.

Figure No 01: Raw Drugs of *Varuna Taila*



METHOD OF PREPARATION OF VARUNA TAILA:

The *Varuna taila* was prepared in GMP-approved well established Parul Ayurveda Pharmacy. Oil was prepared by the reference of

Taila Paka Vidhi mentioned in *Sharangdhar Samhita Madhyam Khanda*.^[9]

Pre-formulation stage (Collection & Authentication): Fresh, authenticated leaves of *Varuna* (*Crateva nurvala*) were collected, cleaned, and processed to obtain *Swarasa* (fresh juice) by grinding and pressing, while *Kalka* (paste) was prepared by wet grinding into a fine, smooth consistency.

Formulation stage (Preparation of Oil): *Tila Taila* (5 Liters) was taken in a wide-mouthed stainless-steel vessel, warmed over *madhyama agni*, and *Varuna Swarasa* (20 Liters) was added gradually with continuous stirring, followed by the addition of *Varuna Kalka* (1.5 kg) as a bolus. The mixture was subjected to *Taila Paka* with regulated heating until *Taila Siddhi Lakshana* were observed.

Post formulation Stage (Storage): After attaining *Siddhi*, the oil was removed from fire, cooled to a tolerable temperature, filtered through a double-layered muslin cloth, and collected into sterilized airtight roll-on bottles, which were labeled and stored in a cool, dry place away from sunlight to preserve stability and therapeutic efficacy.

Precautions taken during preparation of the drug:

- The study was conducted in a laboratory setting, adhering to a preparation-focused design that ensured classical methods of *Tailapaka Vidhi* were followed accurately.
- During *Tailapaka* (oil-cooking), care was taken to maintain moderate and uniform heat, preventing the burning of *Drava Dravya* (herbal decoction or paste) and ensuring proper integration with the oil base.
- Continuous stirring was performed throughout the boiling process, especially toward the end stage, to avoid sticking or scorching of the herbal paste (*Kalka*) and to observe *Madhyama Paka Lakshana*.
- The *Taila Siddhi Lakshanas* (signs of proper preparation) like frothlessness, *Kalka* rolling into a *Varti* (roll), and absence of moisture were carefully observed before stopping the heating.
- After preparation, the *Taila* was filtered through a clean muslin cloth to remove residual *Kalka*.
- The prepared *Taila* was stored in containers after it reached room temperature, protecting it from light and moisture. The containers were labelled and kept in a dry, cool place to preserve potency and shelf life.

Figure no 2: Preparation of *VARUNA TAILA*



Figure 2.1-Fresh juice of *Varuna Patra*



Figure 2.2-*Varuna Patra* Paste in bolus form



Figure 2.3- *Varuna* Fresh Juice & Paste is mixed with warm oil



Figure 2.4- Process of Taila Paka



Figure 2.5-At final Satge of Taila Paka



Figure 2.6-Filtration of Prepared Oil



Figure 2.7 Final Product Varuna taila

RESULT:

ANALYTICAL STUDY-

Macroscopic examination and organoleptic examination of *Varuna Taila* were determined. The physicochemical parameters of the *Varuna Taila* were analysed at pharmaceutical chemistry laboratory of Parul institute of Ayurved

The HPTLC study done at Vasu Research Centre, GIDC, Makarpura, Vadodra (Sample ID- AD/24/252 Dated- 03.08.2024).

Table no.1: Results of organoleptic and Physio-chemical parameters of *Varuna Taila*-

Sr. No.	Parameter	Value
1.	Description Colour Odour Consistency	Oil Yellowish-green Pleasant aromatic smell Liquid
2.	Loss on drying at 110 c (%w/w)	0.09
3.	Total Ash value (%w/w)	0.596
4.	pH	4.3
5.	Specific gravity	1.0901
6.	Viscosity	35.2960
7.	Acid Value	0.8976
8.	Rancidity test	Negative
9.	Refractive index at 400	1.4500
10.	Saponification value	210
11.	Iodine value	97

HPTLC (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

ANALYSIS:

Preparation of Test solution: Take 0.1 mL of Sample in a test tube and dilute it with 1 mL of Hexane. Mix well. Use the Test solution thus obtained for HPTLC fingerprinting.

Preparation of Spray reagent [5 % Sulphuric acid in Methanol reagent]: 5 mL Sulphuric acid is cautiously mixed with 100 mL Methanol.

Table no. 2: Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F254 on Aluminium sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base

Sample Application Volume	7.0 µL
Distance Between Tracks	0 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Petroleum Ether: Diethyl Ether: Acetic Acid (9: 1 : 0.1 v/v)
Pre-chromatographic derivatization	After sample spotting pre-chromatographic derivatization done with 5 % Alcoholic KOH (2.0 µL) followed by heating the plate for 10 minutes on TLC Plate Heater Preheated at 100 ± 50C.
Visualization	@ 254 nm, @ 366 nm (after derivatization) and @ 540 nm (after derivatization)
Spray reagent	5 % Sulphuric acid in Methanol
Derivatization mode	CAMAG - Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 50C for 3 minutes

RESULTS-

Figure no 3: Chromatogram at 254 nm

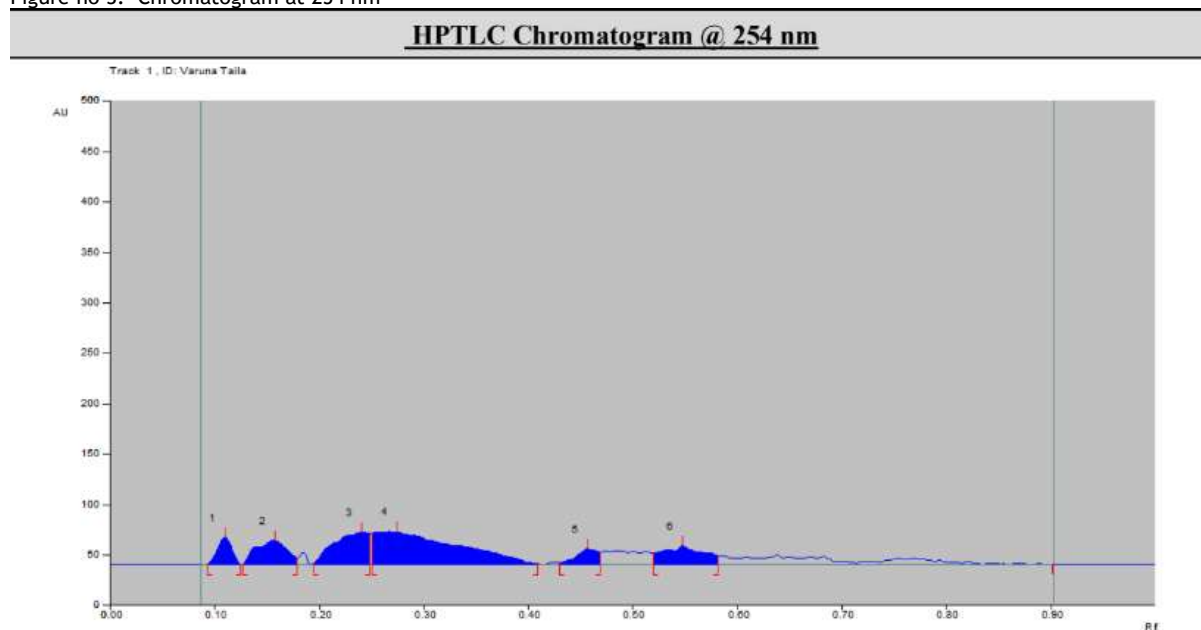
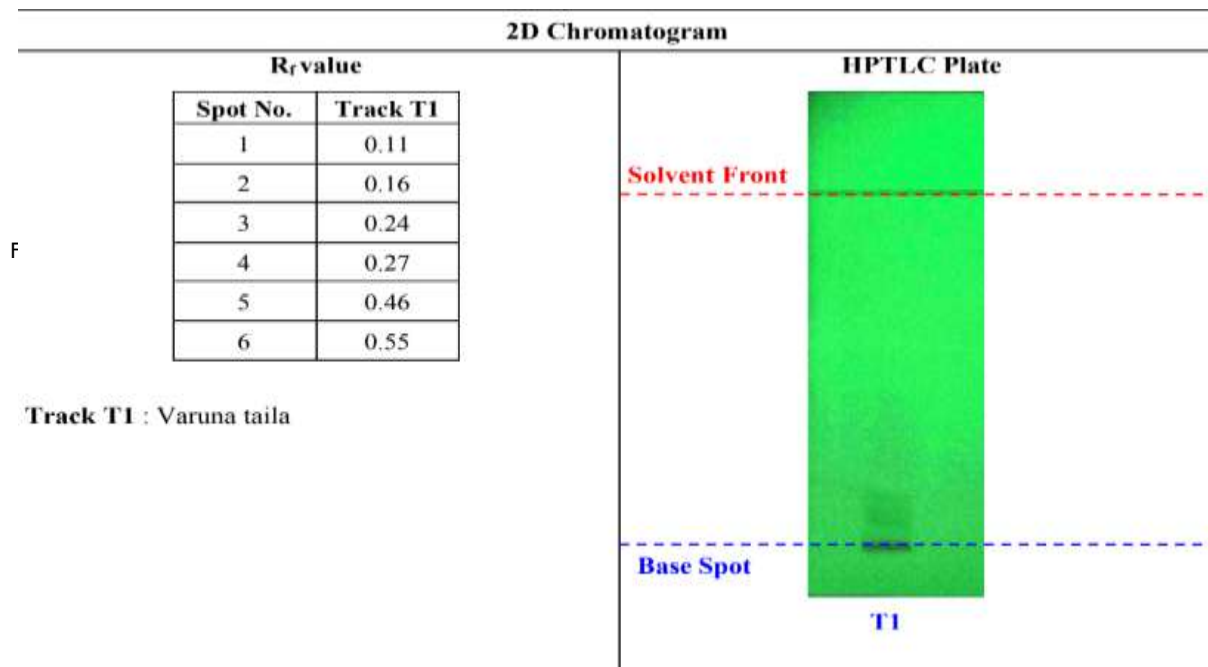


Table no.3: RF

value at 254 nm



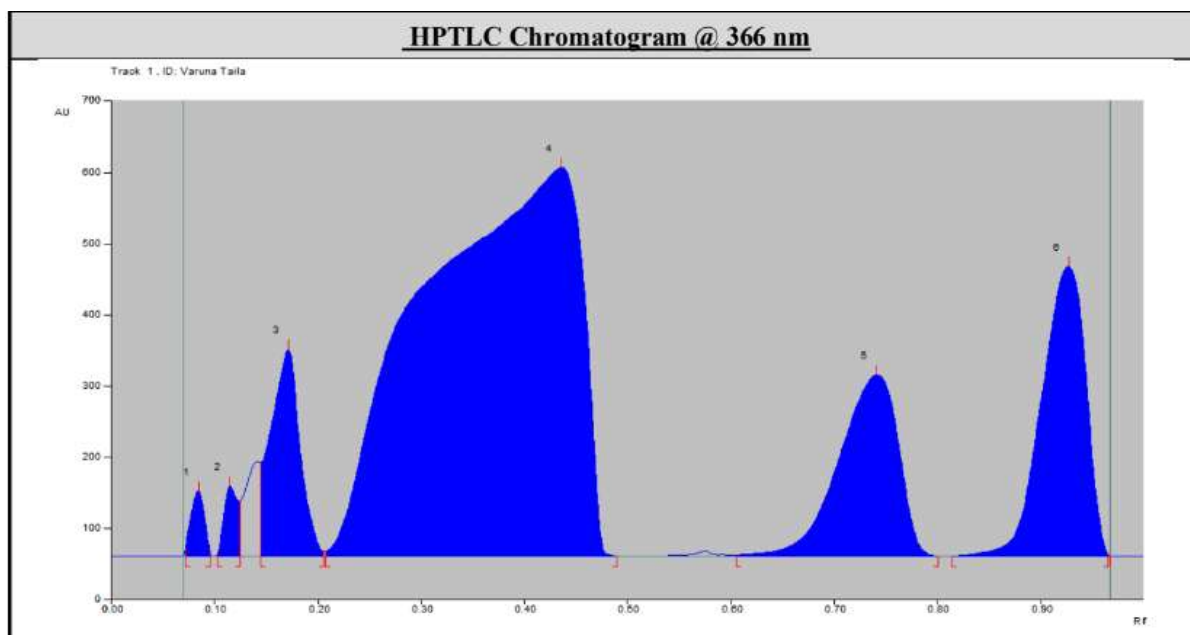


Table no.4: R_f value at 366 nm

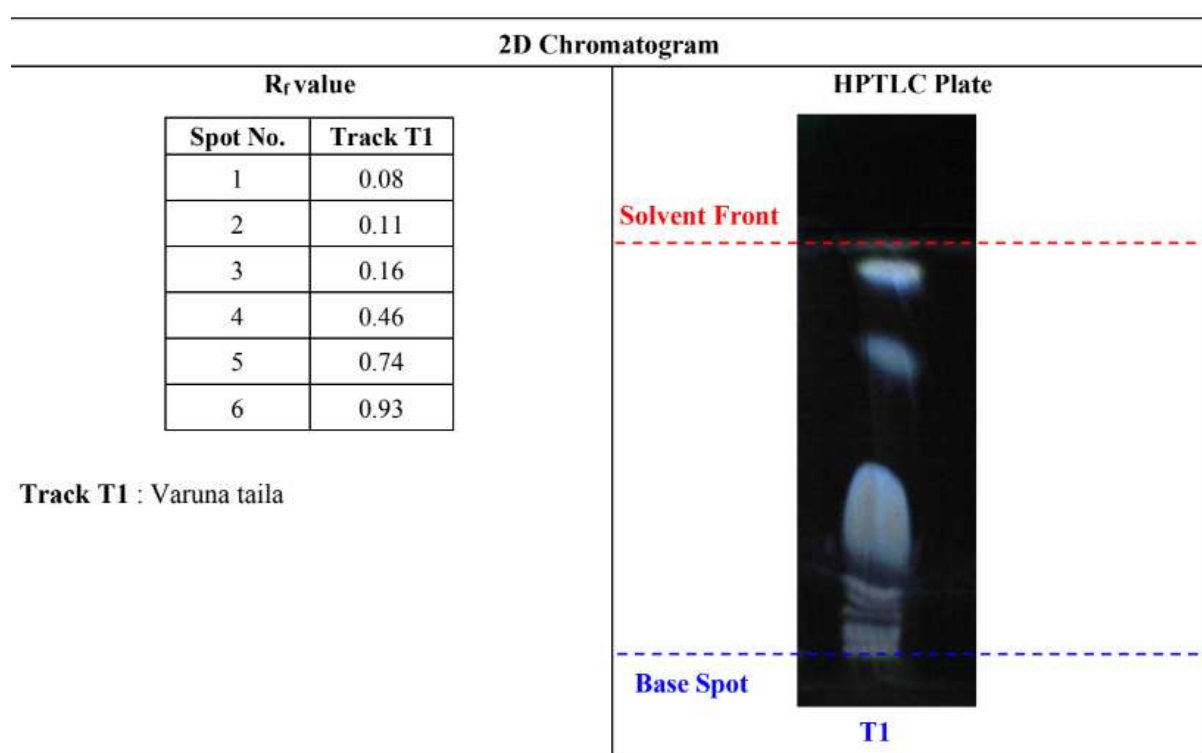


Figure no 5: Chromatogram at 540 nm

HPTLC Chromatogram @ 540 nm

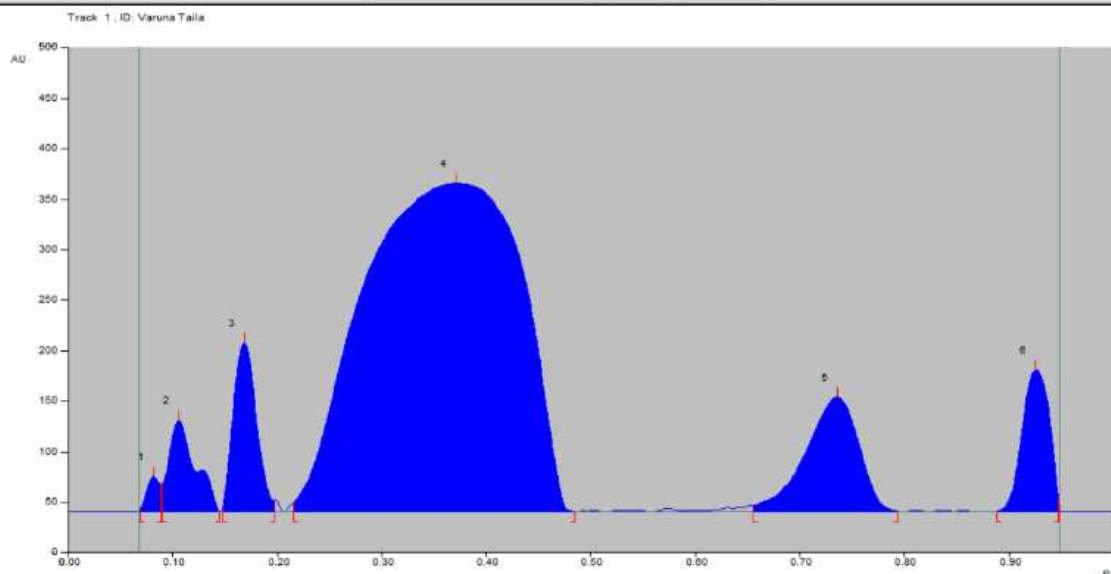


Table no.5: Rf value at 540 nm

2D Chromatogram

R_f value

Spot No.	Track T1
1	0.08
2	0.11
3	0.16
4	0.37
5	0.74
6	0.93

HPTLC Plate

Solvent Front

Base Spot

T1

Track T1 : Varuna taila

DISCUSSION

The present study aimed to standardize and analyse the Ayurvedic formulation *Varuna Taila*, intended for the management of striae gravidarum (*Kikkisa*) during pregnancy. The findings from the physicochemical and HPTLC analyses provide a foundation for establishing the authenticity and quality of this traditional remedy.

HPTLC of *Varuna Taila* was seen at three different wavelengths. At 254 nm, 6 spots were found. At 366nm, 6 spots were found. At 540nm, 6 spots were found. On observing the R_f values, it is seen that three values are common in all the three wavelengths.

According to the pharmaceutical analysis, the *Varuna Taila* is yellowish green in colour. The disintegration time is 64.41 minutes, the pH is 6, and the taste is bitter and astringent. Loss on Drying is 0.09, Total Ash value is 0.596, pH is of 4.3, Refractive

index is 1.4500, Specific gravity is 1.0901, Viscosity is 35.2960, Acid value is 0.8976, Rancidity test is Negative, Saponification value is 210 & Iodine value is 97.

The findings of the HPTLC study of the *Varuna Taila* were obtained in the form of chromatograms (scanned at the wavelengths of 254 nm, 366 nm, and 540 nm) representing various peaks. The HPTLC analysis was performed using the CAMAG Linomat 5 HPTLC machine. The phytochemical profile of the medication was ascertained and is displayed in tables with R_f values, peak area, peak heights, total number of peaks, and percent area. In this study the chemical constituents found according to R_f value are L-Stachydrine, Dodecanac anhydride, Methyl pentacosanoate, Kaemferol-o- α -D glucoside, and Quercitin-3-O- α -D glucoside, suggesting potential benefits for skin elasticity, hydration and

collagen remodelling, which are critical in preventing and managing stretch marks.

As seen in the figures no 3, 4 & 5 and tables no 3, 4 & 5 the HPTLC analysis of the *Varuna Taila* revealed the presence of a number of phytoconstituents at varying amounts. In the *Varuna Taila* High-Performance Thin-Layer Chromatography (HPTLC) examination, the determined retention factor (RF) values show the presence of many substances.

The standardization process followed in this study involved meticulous preparation as per the *Sharangdhara Samhita*, ensuring consistency and safety of the formulation. The physicochemical analysis, including pH, refractive index, specific gravity, provides essential parameters for quality assurance. The HPTLC fingerprinting further establishes a reference profile for *Varuna Taila*, serving as a preliminary tool for future batch-to-batch consistency and authentication^[10].

Recent clinical studies on topical oil formulations containing plant extracts and vitamins have demonstrated improvements in the appearance of stretch marks by enhancing skin hydration, promoting collagen remodelling, and increasing dermal thickness, which together contribute to better elasticity and gradual fading of discoloration.^[12] While these studies use different ingredients, the underlying principle that is the topical application of bioactive compounds to improve skin elasticity and hydration, aligns with the rationale for using *Varuna Taila*^[9]. The phytochemical profile of *Varuna Taila* suggests it may offer similar benefits, though clinical trials specific to this formulation are warranted to confirm efficacy and safety.

CONCLUSION

The present study successfully standardized *Varuna Taila* and established a preliminary HPTLC fingerprint for quality control. The formulation's traditional use, phytochemical profile, and safety profile support its potential as a natural, cost-effective remedy for striae gravidarum in pregnancy.

Funding: Not any

Author Contributions:

Dr. Margi: Drug preparation, Drug analysis, Methodology, Visualization, Review & editing

Dr. Shriniwas: Review & editing, Supervision

Dr. Keya: Drug preparation, Review & editing, Supervision

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Conflict of interest: No conflicts of interest to declare

Declaration of generative AI in scientific writing: The authors utilized ChatGPT for language editing assistance. After using AI, they thoroughly reviewed and refined the content, taking full responsibility for the final publication.

Data Availability statement: All data related to the outcome are included in the manuscript.

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Supplementary material

▪ Drug Identification Certificate

RAW DRUG AUTHENTICATION COMMITTEE PARUL INSTITUTE OF AYURVED



PUI/PIA/DG-Cert-248

Name of the Sample: Varuna Taila

Date- 29/04/2024


CERTIFICATE


This is to certify that Dr. Margi Patel provided the Plant sample.
Scholar, Department of Prasuti Tantra Evam Stree Roga of Parul Institute of Ayurved is that of:

Sr. No.	Name of the sample	Botanical Name	Family	Useful Part
1	Varuna	Crataeva nurvula	Capparidaceae	Leaves

The raw drugs are identified under our committee supervision.

1. <https://indiabiodiversity.org/species/show/251028>


Professor
Dravyaguna Dept.
Parul Institute of Ayurved
Parul University, Vadodara


Associate Professor
Pharmacognosy Dept.
Parul Institute of Pharmacy
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Parul Ayurved Pharmacy

GMP Certified (GA/1842)

Parul University

Limda, Waghodia, Vadodara

Date: 26/06/2024

Certificate

DR. MARGI PATEL, P.G Scholar from the Department of PRASUTI TANTRA EVUM STRI ROGA, Parul Institute of Ayurved, Parul University. She had prepared medicines in GMP certified Parul Ayurved Pharmacy as a part of her dissertation work on "AN OPEN LABELLED RANDOMIZED COMPARATIVE CLINICAL STUDY TO EVALUATE THE EFFICACY OF VARUNA TAILA AND KARANJA TAILA IN THE PREVENTION OF KIKKISA WITH SPECIAL REFERENCE TO STRIAE GRAVIDARUM" Guided by DR. SRINIWAS JADHAV, ASSOCIATE PROFESSOR, DEPARTMENT of PRASUTI TANTRA EVUM STRI ROGA in Parul Ayurved Pharmacy, Parul Institute of Ayurved, Parul University.

Details of Medicines are given below:

Sr.No	Name of Medicine	Reference	REMARK
1	VARUNA TAILA	BHAISHAJYA RATNAVALI	5 LITER
2	KARANJA TAILA	ASHTANGA SAMGRAHA	5 LITER

I hereby certify that the method of preparation was done following the standards.


 Deputy Director
 Dept of RSBK
 DEPUTY DIRECTOR
 PARUL AYURVED PHARMACY




 Director & Principal
 Parul Ayurved Pharmacy
 Director
 Parul Ayurveda Pharmacy

CENTRAL RESEARCH LABORATORY

PARUL INSTITUTE OF AYURVED



CERTIFICATE

This is to certify that Dr. Margi S Patel from the Department of Prasuti Tantra Evam Stri Roga has completed his Quality testing of the finished product in the Central Research Laboratory for the research work entitled "AN OPEN LABELLED RANDOMIZED COMPARATIVE CLINICAL STUDY TO EVALUATE THE EFFICACY OF APPLICATION OF *VARUNA TAILA* AND *KARANJA TAILA* IN THE PREVENTION OF *KIKKISA* W.S.R TO STRIAE GRAVIDARUM"

The work has been conducted under my direct supervision.


Principal
PRINCIPAL
Parul Institute of Ayurved,
Vadodara




Head
Central Research Laboratory
Parul Institute of Ayurved,
Vadodara

**CENTRAL RESEARCH LABORATORY
PARUL INSTITUTE OF AYURVED**

DRUG ANALYSIS REPORT



Drug name	: VARUNA TAILA AND KARANJA TAILA
Manufacturing date	: 26/06/2024
Analysis date	: 05/07/2024
Name of scholar	: Dr. Margi Sureshbhai Patel
Thesis title – “AN OPEN LABELLED RANDOMIZED COMPARATIVE CLINICAL STUDY TO EVALUATE THE EFFICACY OF APPLICATION OF <i>VARUNA TAILA</i> AND <i>KARANJA TAILA</i> IN THE PREVENTION OF <i>KIKKISA</i> W.S.R TO STRIAE GRAVIDARUM”	
Department: Prasuti Tantra Evam Stri Roga	
Year: 2022-2023	

ORGANOLEPTIC CHARACTERISTICS

SAMPLE	<i>VARUNA TAILA</i>	<i>KARANJA TAILA</i>
Color	Yellowish green	Yellowish green
Odor	Pleasant aromatic	Pleasant aromatic
Consistency	Liquid	Liquid

PHYSICO-CHEMICAL PARAMETERS

	SAMPLE	<i>VARUNA TAILA</i>	<i>KARANJA TAILA</i>
Sr. No	PARAMETRE	VALUE	
1.	Loss on Drying at 110 c(%w/w)	0.09	0.47
2.	Total Ash Value (%w/w)	0.596	0.791
3.	P ^H Value	4.3	4.3
4.	Specific gravity	1.0901	1.0750
5.	Viscosity	35.2960	33.02540
6.	Acid Value	0.8976	1.2200
7.	Rancidity test	Negative	Negative
8.	Refractive index	1.4500	1.4480
9.	Saponification value	210	205
10.	Iodine value	97	95

	Analyzed by	Checked by	Approved by
Designations	PG. SCHOLAR	CENTRAL RESEARCH LABORATORY IN CHARGE	PRINCIPAL PARUL INSTITUTE OF AYURVEDA
	M. S. Patel		



HPTLC finger printing

<u>HPTLC FINGERPRINTING REPORT</u>	
Sample	: Varuna Taila
Name of Scholar	: Dr. Margi Patel, Parul Institute of Ayurved, Parul University, Vadodara.
Sample ID	: AD/24/252
Date of Report	: 03.08.2024
Preparation of Test solution: Take 0.1 mL of Sample in a test tube and dilute it with 1 mL of Hexane. Mix well. Use the Test solution thus obtained for HPTLC fingerprinting.	
Preparation of Spray reagent [5 % Sulphuric acid in Methanol reagent]: 5 mL Sulphuric acid is cautiously mixed with 100 mL Methanol.	
Chromatographic Conditions:	
Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F ₂₅₄ on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Sample Application Volume	7.0 µL
Distance Between Tracks	0 mm
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Petroleum Ether : Diethyl Ether : Acetic Acid (9 : 1 : 0.1 v/v)
Pre-chromatographic derivatization	After sample spotting pre-chromatographic derivatization done with 5 % Alcoholic KOH (2.0 µL) followed by heating the plate for 10 minutes on TLC Plate Heater Preheated at 100 ± 5°C.
Visualization	@ 254 nm, @ 366 nm (after derivatization) and @ 540 nm (after derivatization)
Spray reagent	5 % Sulphuric acid in Methanol
Derivatization mode	CAMAG – Dip tank for about 1 minute
Drying Mode, Temp. & Time	TLC Plate Heater Preheated at 100± 5°C for 3 minutes

Analyzed by

Approved by

Officer

Dy. Manager



Vasu Research Centre (A Div. of Vasu Healthcare Pvt. Ltd.)
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