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Research Paper

Quality Control and Shelf-Life Study of Dasmula Taila- A Classical Ayurvedic Formulation for Nasya

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ABSTRACT

Background: In Ayurveda, medicated oils are primarily employed to address ailments related to Vata Dosha imbalance. Dasmula Taila, a classical formulation mentioned in Bhaisajya Ratnavali, is used for managing Shiro-roga (head diseases) and nasya (administration of medicament through nostrils). **Objectives:** **Methods:** The key ingredients include roots of Bilva (*Aegle marmelos* (L.) Corrêa), Shyonaka (*Oroxylum indicum* (L.) Kurz), Gambhari (*Gmelina arborea* Roxb), Patala (*Stereospermum suaveolens* (Roxb.) DC), Agnimantha (*Clerodendrum phlomidis* Linn), Shalaparni (*Desmodium gangeticum* (L.) DC), Prishniparni (*Uraria picta* (Jacq.) Desv), Kantakari (*Solanum xanthocarpum* Schrad. & H. Wend), Brihati (*Solanum indicum* Linn), Goksura (*Tribulus terrestris* L.) and murchit Tila Taila. Rich in Tridosha-calming substances, it is prepared by using the traditional Taila-paka method, a form of oil preparation detailed in ancient Ayurvedic texts, and adheres to the prescribed procedures outlined in the Ayurvedic Pharmacopoeia of India (API). The organoleptic study and physicochemical analysis have provided valuable information towards the development of quality control data of the formulation. Also the chemical standards of the formulation were developed using sophisticated analytical techniques like high performance thin layer chromatography (HPTLC) study. **Results:** As no detail study on the quality control parameters of Dasmula Taila is available in existing literature, this study is essential to establish quality control benchmarks for the prepared Dasmula Taila formulation. In this regard Quality control data of three batches of Dasmula Taila was prepared along with HPTLC profiling. Accelerated shelf life study was also carried out to access its stability and the data suggests that the formulation is stable for at least for 6 months.

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Conclusion: The current research findings establish the quality control benchmarks for the raw materials used, which will contribute to developing the quality criteria for the Dasmula Taila formulation. This also includes creating an HPTLC fingerprint profile for the Dasmula Taila formulation. Besides this, stability studies of Taila have also been performed.

INTRODUCTION

Ayurveda is a science of life and it is the ancient and comprehensive medical system, focuses on holistic healing using natural remedies, including herbal formulations and purification procedures [1]. In Ayurveda, *Dash* stands for ten (10) and *Moola* stands for Root. *Dasmula*, a combination of *Brihat Panchamoola* and *Laghu Panchamoola*, contains ten roots of ten different plants in equal ratio. Out of the ten roots five roots are of trees, known as *Brihat Panchmoola* and five roots are of shrubs, known as *Laghu panchmoola*. *Brihat Panchmoola* contains *Bilva* (*Aegle marmelos* (L.) Corrêa), *Shyonaka* (*Oroxylum indicum* (L.) Kurz), *Gambhari* (*Gmelina arborea* Roxb), *Patala* (*Stereospermum suaveolens* (Roxb.) DC) and *Agnimantha* (*Clerodendrum phlomidis* Linn) whereas *Laghu Panchmoola* contains *Shalaparni* (*Desmodium gangeticum* (L.) DC), *Prishniparni* (*Uraria picta* (Jacq.) Desv), *Kantakari* (*Solanum xanthocarpum* Schrad. & H. Wend), *Brihati* (*Solanum indicum* Linn) and *Goksura* (*Tribulus terrestris* L.) [2]. Ayurveda makes extensive use of a mixture of these ten (10) roots, which work on *Vata* and *Dosha* to lessen their aggravation. There are a plethora of health benefits associated with *Dasmula*, gout, Parkinson's disease, arthritis, asthma, headaches, puerperal disorders, muscle spasms, lower back aches are the some of those. It has strong analgesic, antioxidant, and anti-inflammatory effects [3-4]. In the Ayurvedic system, health is defined by the equilibrium of *Dosha*, *Dhatu*, and *Malas*, which are the body's regulatory, structural, and waste-removal components [4]. The polyherbal combination is one of the most often used

ingredients in Ayurveda medicine, where it is utilized to make various kinds of medicine that are used to treat different disorders. *Dasmula Taila* is a herbal oil blend prescribed for *Nasya* treatment in cases of *Shiro-roga*, a category of head-related ailments [2-3]. Sharma *et al.* recently conducted a clinical study on the *Dasmula Taila Nasya* and *Shirodhara* in *Vatika Shiroroga* (Tension Headache) [5] which showed that both therapies were approximately equally beneficial, but patients who were administered *Shirodhara* with *Dashmoola Taila* showed better results in comparison to those administered *Nasya Karma* with *Dasmula Taila*. This preparation is also linked to treating *Ardhambhedaka*, which is comparable to migraines. The *Taila* formulation is crafted to harmonize the body's elements, specifically to soothe *Vatadosha* without worsening *Kapha* [6]. During its preparation, known as *Snehpaka*, *Taila* uniquely integrates the qualities of the herbs infused into it while retaining its inherent characteristics [7]. This study chooses *Dasmula Taila* for managing *Ardhambhedaka* due to its predominantly *Tridoshamaka* contents.

MATERIALS AND METHODS

Plant Materials

In the present study, roots of the ten (10) selected plants roots viz. *Bilva* (*Aegle marmelos* (L.) Corrêa), *Shyonaka* (*Oroxylum indicum* (L.) Kurz), *Gambhari* (*Gmelina arborea* Roxb), *Patala* (*Stereospermum suaveolens* (Roxb.) DC), *Agnimantha* (*Clerodendrum phlomidis* Linn), *Shalaparni* (*Desmodium gangeticum* (L.) DC), *Prishniparni* (*Uraria picta* (Jacq.) Desv), *Kantakari* (*Solanum xanthocarpum* Schrad. & H. Wend), *Brihati* (*Solanum indicum* Linn), *Goksura* (*Tribulus terrestris* L.) was collected [2]. The selected plant roots were authenticated by the Pharmacognosy department of CARI, Kolkata, along with macroscopical and powder microscopical characterization as per API. [8-9]



Preparation of Dasmula Taila

The oil 'Dasmula Taila' was prepared by the roots of ten (10) plants along with *Tila taila* (sesame oil), which was chemically standardized by testing of the parameters, *i.e.* Acid value, Saponification value, Unsaponifiable matter, Iodine value, Peroxide value *etc.* The physicochemical parameters (Loss on drying, Total ash, Acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH, HPTLC) and safety parameters (Heavy metals, microbial load, specific pathogens, pesticide residue testing) analysis were carried out for all the mentioned raw drugs as per API (Table 1). The *murchit tila taila* was obtained by following the *murchana* process of *Tila taila* using the raw drugs *viz.* *Manjistha* (Root), *Musta* (Rz.), *Tvak/Nalika* (St. Bk.), *Haritaki* (Fr.), *Haridra* (Rz), *Ketaki* (Root), *Bibhitaka* (Fr.), *Amalaki* (Fr.), *Hriversa* (Root), *Vatankura* (St. Bk.) and *Lodhra* (St. Bk.) which were macroscopically studied and chemically standardized as per API [10, 11]. At first, all the plant roots were cleaned, washed, dried and powdered separately. After that, all powdered raw drugs were passed individually through a sieve size 80. After that, all these raw drugs, 320 gm each, were taken, and 16 litres of water was added to make a homogeneous mixture. The process began by adding 1 litre *Murchit Tila Taila* to the mixture and heating it at a temperature range of 50-60 °C for three hours. The heat was turned off once the three hours had elapsed, and the boiler was left to rest overnight. The next day, heating resumed, and froth development was monitored while regularly checking the *kalka* for *varti* formation until the froth eventually settled. Subsequently, the *varti* was strained while still hot (around 80 °C) through a muslin cloth. After cooling, the clear liquid collected was the final *Dasmula Taila* product.

Physicochemical ananlysis

The powder form of each plant part were used for the physicochemical analysis as per the WHO/API guidelines. For determining the water and alcohol extractive values, the crude powder (4 g) was soaked in the respective solvents (100 mL) with occasional shaking for up to 6 h, followed by keeping the mixture overnight (18 h) at room temperature. The extracts were subsequently concentrated in a hot water bath to obtain constant weights, and the extractive values were calculated. In order to determine Loss on drying, the crude powder (4 g) was kept in hot air oven at 105 °C for 6 h, followed by cooling weight is noted and LOD at 105 °C was calculated. For total ash determination, 2 g of each raw drugs (powder form) were taken in a silica crucible and incinerated at 450 °C for 6 h. On the next day the weight of the crucible is noted and result is calculated. Now the ash is ready for determination of acid insoluble ash. The ash obtained on the previous day was treated with 25 mL 2(N) HCl and warmed in a water bath, filtered through a ashless filter paper. Once all the liquid were drained, the ashless filter paper was transferred in the same silica crucible and incinerated at 450 °C for 6 h. On the next day the weight of the crucible is noted and result is calculated. In order to check the pH, 10 % aqueous suspension was prepared, kept for 24 h and pH was noted. The formulation *Damula Taila* is liquid and three (03) batches of formulations have been prepared. Physicochemical investigations *viz.* determination of Refractive index at 40 °C, Viscosity, Specific optical rotation, Acid value, Iodine value, Saponification value, Unsaponifiable matter, Peroxide value, Specific gravity and Wt per ml (at 25 °C) of the three batches of *Dasmula Taila* were carried out following API methods. [12-13]

High Performance Thin Layer Chromatography (HPTLC) study [14-15]
Equipment



A CAMAG HPTLC system (Switzerland), comprising of a CAMAG ATS 4, CAMAG TLC scanner 3, CAMAG Wincats Software, Version 1.44, a CAMAG TLC plate heater, and a CAMAG Visualizer, was used for the study.

Preparation of samples

(a) *Murchit Tila Taila* (oil) along with raw drugs used for Murchana

For each sample, 1 gram was refluxed with 20 ml of Methanol for one hour. The resulting extracts were then passed through filter paper. The filtrates obtained were concentrated for Thin Layer Chromatography (TLC) analysis.

(b) *Dasmula Taila* along with its ten (10) raw ingredients and markers

The High-Performance Thin Layer Chromatography (HPTLC) analysis of the *Dasmula Taila* formulation and the raw materials and reference markers was conducted across three separate batches. Each sample, weighing 1 gram (or 2 mL for *Taila*), underwent reflux with 20 mL of Methanol for one hour; post-reflux, the solution was filtered through filter paper. The resulting filtrate was then reduced in volume and prepared for TLC analysis. Reference standards were created by dissolving 1 mg each of marmelosin, beta-sitosterol, and lupeol acetate in 10 mL of Methanol.

Stationary phase

Pre-activated 20 × 10 cm aluminum-supported precoated silica gel 60 F₂₅₄ plates (Merck, India, Batch No. 1.05554.0007) were used.

Application of samples

A 5 µL sample was applied to the plate as an 8 mm band, positioned 15 mm above the plate's base using CAMAG ATS4 applicator.

Mobile phase

(c) *Murchit Tila Taila* (oil) along with raw drugs used for Murchana

The solvent system used was a mixture of Toluene, Ethyl acetate, and Formic acid in the ratio of 7:3:0.1(v/v).

(d) *Dasmula Taila* along with its ten (10) raw ingredients and markers

The solvent system comprised a blend of toluene, ethyl acetate, and formic acid in a 7.5:2.5:0.4 (v/v) ratio.

Plate development and detection of spots

The plates were developed to a distance of 90 mm in a CAMAG Twin trough chamber, with a pre-conditioning period of 20 minutes at 25°C and 42% average relative humidity. 20% aqueous sulphuric acid solution was used as the derivatising agent. The TLC plate was developed up to 90 mm in a pre-saturated CAMAG twin trough chamber after preconditioning the plate at 25 °C and relative average humidity of 42%. Images of the developed plate were captured at 254, 366 nm and in white light.

Shelf life study [16-17]

The stability of the formulation was evaluated in a stability chamber (Model: REMI 12C) under regulated conditions (temperature at 38 °C and relative humidity at 75%) over a period of six months, with assessments at intervals of one month (01), three months (03), and six months (06). The stability profile, composed of specific parameters, is essential for determining appropriate storage conditions and the expiration period for pharmaceuticals. The construction of the stability study should rely on an understanding of the drug's characteristics and the physical form in which it is administered. The purpose of the present study was to establish stability data by taking three batches of drug formulation at specified intervals of 0 days, 1st month, 3rd month, and 6th month. Various physical parameters, like the refractive index, viscosity, and specific gravity, as well as chemical parameters, like saponification, acid, and iodine values, were studied.

RESULT AND DISCUSSION

Organoleptic and quality control studies carried out on the ingredients of *Dasmula Taila*

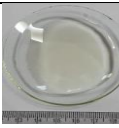
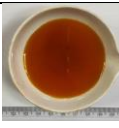


In *Dasmula Taila* the ingredients present are the roots of the following ten (10) plants viz. *Bilva* (*Aegle marmelos* (L.) Corrêa), *Shyonaka* (*Oroxylum indicum* (L.) Kurz), *Gambhari* (*Gmelina arborea* Roxb), *Patala* (*Stereospermum suaveolens* (Roxb.) DC), *Agnimantha* (*Clerodendrum phlomidis* Linn), *Shalaparni* (*Desmodium gangeticum* (L.) DC),

Prishniparni (*Uraria picta* (Jacq.) Desv), *Kantakari* (*Solanum xanthocarpum* Schrad. & H. Wend), *Brihati* (*Solanum indicum* Linn), *Goksura* (*Tribulus terrestris* L.) and *Murchit Tila Taila*. In The following table (Table 1) photographs, organoleptic findings, physicochemical parameters and safety parameters of the ingredients of *Dasmula Taila* are documented.

Table 1: List of ingredients of Dasmula Taila along with their photographs and quality control data

Sl. No.	Plant Name	Plant part used	Photograph	Organoleptic study	Physicochemical Parameters (% w/w)	Safety Parameters	Sl. No.	Plant Name	Plant part used
				Colour	Texture	Odour			
1	<i>Bilva</i> [<i>Aegle marmelos</i> (L.) Corrêa] [18] (API, Part-I, Vol-III, Pg. 29-31)	Root		Greyish brown	Rough	Faintly aromatic	1	<i>Bilva</i> [<i>Aegle marmelos</i> (L.) Corrêa] [18] (API, Part-I, Vol-III, Pg. 29-31)	Root
2	<i>Shyonaka</i> [<i>Oroxylum indicum</i> (L.) Kurz] [19] (API, Part-I, Vol-III, Pg. 209-210)	Root		Light brown	Rough	Characteristic	2	<i>Shyonaka</i> [<i>Oroxylum indicum</i> (L.) Kurz] [19] (API, Part-I, Vol-III, Pg. 209-210)	Root
3	<i>Gambhari</i> [<i>Gmelina arborea</i> Roxb] [20] (API, Part-I, Vol-I, Pg. 36-37)	Root		Orangish brown	Rough	Not characteristic	3	<i>Gambhari</i> [<i>Gmelina arborea</i> Roxb] [20] (API, Part-I, Vol-I, Pg. 36-37)	Root
4	<i>Patala</i> [<i>Stereospermum suaveolens</i> (Roxb.) DC] [21] (API, Part-I, Vol-III, Pg. 147-148)	Root		Orangish brown	Rough	No odour	4	<i>Patala</i> [<i>Stereospermum suaveolens</i> (Roxb.) DC] [21] (API, Part-I, Vol-III, Pg. 147-148)	Root

5	<i>Agnimantha</i> [<i>Clerodendrum phlomidis</i> Linn] [22] (API, Part-I, Vol-III, Pg. 3-4)	Root		Buff	Rough	Faintly aromatic	5	<i>Agnimantha</i> [<i>Clerodendrum phlomidis</i> Linn] [22] (API, Part-I, Vol-III, Pg. 3-4)	Root
6	<i>Shalaparni</i> [<i>Desmodium gangeticum</i> (L.) DC] [23] (API, Part-I, Vol-III, Pg. 178-180)	Root		Brown	Rough	Not characteristic	6	<i>Shalaparni</i> [<i>Desmodium gangeticum</i> (L.) DC] [23] (API, Part-I, Vol-III, Pg. 178-180)	Root
7	<i>Prishniparni</i> [<i>Uraria picta</i> (Jacq.) Desv] (Not in API)	Root		Orangish brown	Rough	Not characteristic	7	<i>Prishniparni</i> [<i>Uraria picta</i> (Jacq.) Desv] (Not in API)	Root
8	<i>Kantakari</i> [<i>Solanum xanthocarpum</i> Schrad. & H. Wendl] (Not in API)	Root		Yellowish brown	Rough	Nil	8	<i>Kantakari</i> [<i>Solanum xanthocarpum</i> Schrad. & H. Wendl] (Not in API)	Root
9	<i>Brihati</i> [<i>Solanum indicum</i> Linn] [24] (API, Part-I, Vol-II, Pg. 27-28)	Root		Greenish brown	Rough	Faint	9	<i>Brihati</i> [<i>Solanum indicum</i> Linn] [24] (API, Part-I, Vol-II, Pg. 27-28)	Root
10	<i>Goksura</i> [<i>Tribulus terrestris</i> L.] [25] (API, Part-I, Vol-I, Pg. 40)	Root		Greenish brown	Rough	Slightly aromatic	10	<i>Goksura</i> [<i>Tribulus terrestris</i> L.] [25] (API, Part-I, Vol-I, Pg. 40)	Root
11	<i>Tila Taila</i> (Sesamum oil)	Oil		Colorless	Oily	Odorless	11	<i>Tila Taila</i> (Sesamum oil)	Oil
12	<i>Murchit Tila Taila</i>	Oil		Light brown	Oily	Mild, characteristic	12	<i>Murchit Tila Taila</i>	Oil

Abbreviations: LOD- Loss on drying; TAV: Total ash value; AIA: Acid insoluble ash; WSE- Water soluble extractive; ASE- Alcohol soluble extractive

HPTLC study of *Murchit Tila Taila* (oil) along with raw drugs used for Murchana

The *murchit tila taila* was obtained by following the *murchana* process of *Tila taila* using the raw drugs viz. *Manjistha* (Root), *Musta* (Rz.), *Tvak/Nalika* (St. Bk.), *Haritaki* (Fr.), *Haridra* (Rz), *Ketaki* (Root), *Bibhitaka* (Fr.), *Amalaki* (Fr.), *Hriversa* (Root), *Vatankura* (St. Bk.) and *Lodhra* (St. Bk.). For each sample, 1 gram was refluxed with 20 ml of Methanol for one hour. The resulting extracts were then passed through

filter paper. The filtrates obtained were concentrated for Thin Layer Chromatography (TLC) analysis. The TLC was performed on pre-coated Silica Gel Plates (60 F254) supported on aluminium sheets from Merck. The solvent system used was a mixture of Toluene, Ethyl acetate, and Formic acid in the ratio of 7:3:0.1(v/v). A 5 µL sample was applied to the plate as an 8 mm band, positioned 15 mm above the plate's base. The plates, measuring 20x10cm, were developed to a length of 90 mm in a CAMAG Twin trough chamber, with preconditioning at 25 °C and 42% relative humidity. Figure 1 documents the TLC plates scanned at 254 nm and 366 nm while Table 2 lists the R_f values.

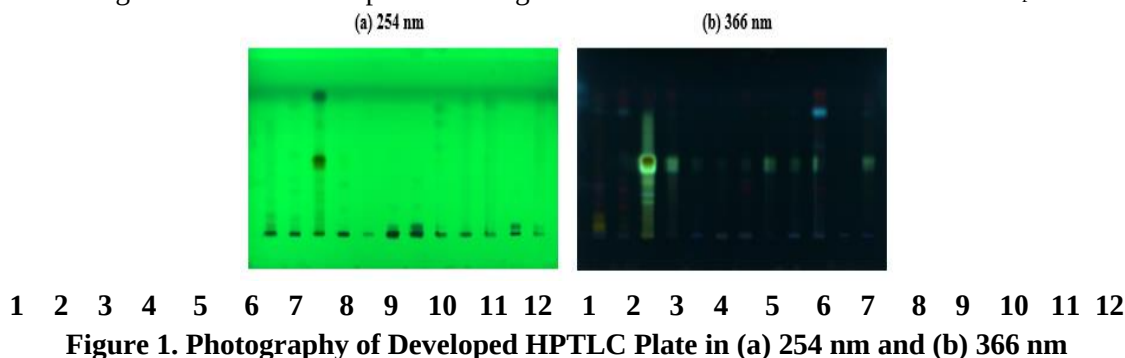


Figure 1. Photography of Developed HPTLC Plate in (a) 254 nm and (b) 366 nm

Table 2: R_f values

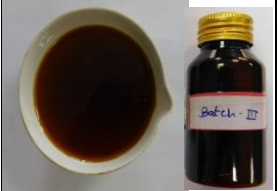
Track No.	Sample name	R_f values (Observed at 254nm)	R_f values (Observed at 366nm)
1.	<i>Manjistha</i> (Rt.)	0.04,0.12,0.18,0.61,0.67	0.07,0.12,0.19,0.31,0.36, 0.65,0.73,0.78,0.83
2.	<i>Ketaki</i> (Rt.)	0.04,0.09,0.25,0.32,0.46, 0.73	0.06,0.11,0.17,0.21,0.27, 0.73
3.	<i>Haridra</i> (Rz.)	0.05,0.09,0.19,0.24,0.28, 0.32,0.40,0.44,0.64,0.75,0.82	0.10,0.20,0.25,0.31,0.39, 0.45,0.55,0.65
4.	<i>Lodhra</i> (St. Bk.)	0.14,0.29,0.43	0.18,0.25,0.33,0.39,0.44
5.	<i>Musta</i> (Rz.)	0.12,0.41,0.46	0.32,0.41.
6.	<i>Haritaki</i> (Fr.)	0.04,0.16	0.32, 0.40
7.	<i>Bibhitaki</i> (Fr.)	0.05,0.16,0.32	0.27,0.33,0.40
8.	<i>Tvak</i> (St. Bk.)	0.04,0.51,0.65,0.73	0.41,0.45
9.	<i>Hriversa</i> (Rt.)	0.03,0.33,0.44,0.79	0.41,0.45
10.	<i>Vatnkura</i> (St. Bk.)	0.04,0.44,0.76,0.80	0.18,0.28,0.40,0.46,0.53, 0.73,0.83
11.	<i>Amlaki</i> (Fr.)	0.04,0.15	0.32
12.	<i>Murchit Tila Taila</i>	0.04,0.12, 0.61,0.80	0.29,0.40,0.45,0.61,0.71, 0.82

Organoleptic studies of *Dasmula Taila* (oil)

The *Dasmula Taila* formulation was prepared by the Pharmacy department of CARI, Kolkata, in three

batches (Batch-I, II & III). Organoleptic characteristics, macroscopical analysis and photographs of three batches of *Dasmula Taila* are listed in Table 3.

Table 3: Organoleptic characteristics and photographs of three batches of Dasmula Taila

Batch	Organoleptic study				Photograph
	Colour	Texture	Odour	Taste	
I	Brown	Oily	Mild, characteristic	Tasteless	
II	Brown	Oily	Mild, characteristic	Tasteless	
III	Brown	Oily	Mild, characteristic	Tasteless	

Chemical Standardization of *Dasmula Taila*

The chemical standardization of *Dasmula Taila* was performed by analysing the following parameters, i.e. determination of Refractive index at 40 °C, Viscosity, Specific optical rotation, Acid value, Iodine value, Saponification value, Unsaponifiable matter, Peroxide value, Specific gravity and Wt per ml (at 25 °C). Safety

parameters i.e. testing of heavy metals, aflatoxins, microbial load, test for specific pathogens and pesticides residue were also carried out. Comparative marker-based HPTLC fingerprinting profile of all ten (10) raw drugs & three (03) batches of the formulation were also carried out. All these results are documents in Table 4-9.

Table 4: Physicochemical parameters

Parameters	Result (in w/w%)			Limit (As per API)
	Batch-I	Batch-II	Batch-III	
Refractive index at 40 °C	1.4670	1.4665	1.4670	Not mentioned in API
Viscosity	86.91	82.73	83.51	
Specific optical rotation	+0.50	+0.50	+0.50	
Acid value	1.37	1.46	1.32	
Iodine value	98.67	104.53	100.05	
Saponification value	186.76	196.60	191.83	
Unsaponifiable matter	1.20	1.22	1.25	
Peroxide value	NIL	NIL	NIL	
Specific gravity	0.9196	0.9192	0.9198	

Wt per ml (at 25 °C)	0.910 g	0.910 g	0.910 g	
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Table 5: Heavy metals testing:

Tests	Protocol	Results (mg/kg)			Acceptance Limit (in ppm)
		Batch-I	Batch-II	Batch-III	
Lead	API	0.39	0.47	0.50	10
Arsenic	API	B.L.Q (0.1)	B.L.Q(0.1)	B.L.Q(0.1)	3.0
Cadmium	API	B.L.Q (0.1)	B.L.Q(0.1)	B.L.Q(0.1)	0.3
Mercury	API	B.L.Q (0.1)	B.L.Q(0.1)	B.L.Q(0.1)	1.0

*^{1,2,3} Limit of Detection = < 0.02 ppm)**Table 6: Aflatoxins:**

Parameter	Result			Permissible Limit as in API (in ppb)
	Batch-I	Batch-II	Batch-III	
B ₁	N.D.	N.D.	N.D.	0.5
B ₂	N.D.	N.D.	N.D.	0.1
G ₁	N.D.	N.D.	N.D.	0.5
G ₂	N.D.	N.D.	N.D.	0.1

Table 7: Microbial Load:

Tests	Results (in cfu/gm)			Permissible Limit as in API (in cfu/gm)
	Batch-I	Batch-II	Batch-III	
Total Microbial plate Count	<10	<10	<10	<10 ⁵
Yeast and mould count	<10	<10	<10	<10 ³

Table 8: Pathogens testing:

Tests	Protocol	Results			Acceptance Limit
		Batch-I	Batch-II	Batch-III	
Escherichia coli	As per A.P.I	Abs/gm	Abs/gm	Abs/gm	Abs/gm
Salmonella spp.	As per A.P.I	Abs/gm	Abs/gm	Abs/gm	Abs/gm
Staphylococcus aureus	As per A.P.I	Abs/gm	Abs/gm	Abs/gm	Abs/gm
Pseudomonas	As per A.P.I	Abs/gm	Abs/gm	Abs/gm	Abs/gm

*Abs- Absent

Table 9: Pesticide Residue:

Substance (s)	Result ^s (in ppm)			Permissible Limit prescribed in API (in ppm)
	Batch-I	Batch-II	Batch-III	Batch-I
Alachlor	B.L.Q	B.L.Q	B.L.Q	0.02
Aldrin and Dieldrin (sum of)	B.L.Q	B.L.Q	B.L.Q	0.05
Azinphos-methyl	B.L.Q	B.L.Q	B.L.Q	1.0
Bromopropylate	B.L.Q	B.L.Q	B.L.Q	0.3
Chlordane(sum of cis-,Trans- and Oxythlordane)	B.L.Q	B.L.Q	B.L.Q	0.05
Chlorfenvinphos	B.L.Q	B.L.Q	B.L.Q	0.5
Chlorpyrifos	B.L.Q	B.L.Q	B.L.Q	0.2
Chlorpyrifos-methyl	B.L.Q	B.L.Q	B.L.Q	0.1
Cypermethrin (and Isomers)	B.L.Q	B.L.Q	B.L.Q	1.0
DDT (sum of p,p-DDT, o,p-DDT, p,p-DDE and p,p-TDE)	B.L.Q	B.L.Q	B.L.Q	1.0
Deltamethrin	B.L.Q	B.L.Q	B.L.Q	0.5
Diazinon	B.L.Q	B.L.Q	B.L.Q	0.5
Dichlorvos	B.L.Q	B.L.Q	B.L.Q	1.0
Dithiocarbamates (as CS ₂)	B.L.Q	B.L.Q	B.L.Q	2.0
Endosulfan (sum of isomers and endosulfan sulphate)	B.L.Q	B.L.Q	B.L.Q	3.0
Endrin	B.L.Q	B.L.Q	B.L.Q	0.05
Ethion	B.L.Q	B.L.Q	B.L.Q	2.0
Fenitrothion	B.L.Q	B.L.Q	B.L.Q	0.5
Fenvalerate	B.L.Q	B.L.Q	B.L.Q	1.5
Fonofos	B.L.Q	B.L.Q	B.L.Q	0.05
Heptachlor (sum of Heptachlor and Heptachlorepoxyde)	B.L.Q	B.L.Q	B.L.Q	0.05
Hexachlorbenzene	B.L.Q	B.L.Q	B.L.Q	1.0
Hexachlorocyclohexane isomers (other than γ)	B.L.Q	B.L.Q	B.L.Q	0.3
Lindane (γ -Hexachlorocyclohexane)	B.L.Q	B.L.Q	B.L.Q	0.6
Malathion	B.L.Q	B.L.Q	B.L.Q	1.0
Methidathion	B.L.Q	B.L.Q	B.L.Q	0.2
Parathion	B.L.Q	B.L.Q	B.L.Q	0.5
Parathion-methyl	B.L.Q	B.L.Q	B.L.Q	0.2
Permethrin	B.L.Q	B.L.Q	B.L.Q	1.0
Phosalone	B.L.Q	B.L.Q	B.L.Q	0.1
Piperonyl Butoxide	B.L.Q	B.L.Q	B.L.Q	3.0
Pirimiphos-methyl	B.L.Q	B.L.Q	B.L.Q	4.0
Pyrethrins (sum of)	B.L.Q	B.L.Q	B.L.Q	3.0

Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophnyl sulphide)	B.L.Q	B.L.Q	B.L.Q	1.0
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***Dasmula Taila* along with its ten (10) raw ingredients and markers**

The High-Performance Thin Layer Chromatography (HPTLC) analysis of three batches of *Dasmula Taila* formulation and the raw materials and reference markers was conducted across three separate batches. Each sample, weighing 1 gram (or 2 mL for Taila), underwent reflux with 20 mL of Methanol for one hour; post-reflux, the solution was filtered through filter paper. The resulting filtrate was then reduced in volume and prepared for TLC analysis. Reference standards were created by dissolving 1 mg each of marmelosin, beta-sitosterol, and lupeol acetate in

10 mL of Methanol. For the chromatography, precoated Silica Gel Plates (60 F254) on aluminium sheets supplied by Merck were utilized. The solvent system comprised a blend of toluene, ethyl acetate, and formic acid in a 7.5:2.5:0.4 ratio. The plant extracts, 5 μ L each, were applied to the plate as 8 mm bands, placed 15 mm from the bottom edge. The plates, sized 5x10 cm, were developed to a distance of 90 mm in a CAMAG Twin trough chamber, with a preconditioning period of 20 minutes at 25°C and 42% average relative humidity. The chemical development was completed using a 20% aqueous sulphuric acid solution as the derivatising agent.

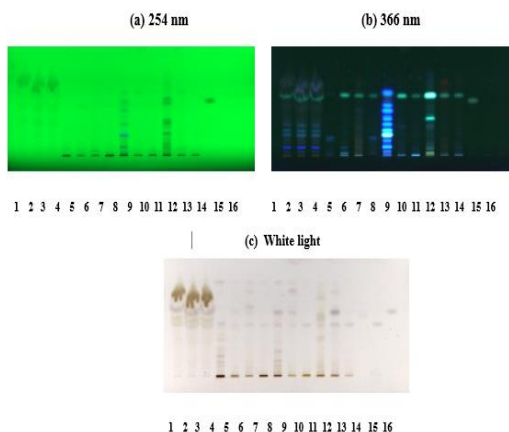


Figure 2. Photography of Developed HPTLC Plate in (a) 254 nm, (b) 366 nm and (c) white light

Table 10. R_f values

Track No.	Sample name	R _f values (Observed at 254 nm)	R _f values (Observed at 366 nm)	R _f values (Observed at white light)
1	<i>Dasmula</i> Formulation Batch-I	0.33, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.45, 0.48, 0.58, 0.71	0.47, 0.53, 0.61, 0.75

2	<i>Dasmula</i> Formulation Batch-II	0.32, 0.47, 0.68	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.47, 0.54, 0.64	0.45, 0.51, 0.57, 0.71
3	<i>Dasmula</i> Formulation Batch-III	0.32, 0.47, 0.68	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.48, 0.55, 0.65	0.45, 0.51, 0.58, 0.70
4	<i>Shalaparni</i> (Rt.)	0.07	0.15, 0.18, 0.61	0.05, 0.09, 0.13, 0.18, 0.22, 0.24, 0.34, 0.39, 0.45, 0.49, 0.86
5	<i>Agnimantha</i> (Rt.)	0.07, 0.48	0.03, 0.09, 0.24, 0.30, 0.37, 0.60	0.45, 0.60, 0.87
6	<i>Goksura</i> (Rt.)	0.05	0.03, 0.08, 0.09, 0.13, 0.17, 0.21, 0.28, 0.33, 0.38, 0.45, 0.59	0.04, 0.07, 0.47, 0.58, 0.65, 0.77, 0.86
7	<i>Brishniparni</i> (Rt.)	0.02	0.16, 0.18, 0.59	0.04, 0.47, 0.86
8	<i>Bilva</i> (Rt.)	0.08, 0.19, 0.31, 0.44, 0.53, 0.62	0.02, 0.04, 0.09, 0.15, 0.21, 0.25, 0.31, 0.36, 0.39, 0.44, 0.50, 0.55, 0.62	0.07, 0.10, 0.14, 0.23, 0.32, 0.47, 0.59, 0.86
9	<i>Gambhari</i> (Rt.)	0.63	0.08, 0.14, 0.21, 0.29, 0.59	0.47, 0.58, 0.64, 0.79, 0.86
10	<i>Brihati</i> (Rt.)	0.06	0.08, 0.21, 0.25, 0.29, 0.59	0.47
11	<i>Patala</i> (Rt.)	0.08, 0.11, 0.13, 0.15, 0.22, 0.32, 0.36, 0.52, 0.57, 0.64, 0.70	0.03, 0.07, 0.08, 0.12, 0.27, 0.30, 0.37, 0.41, 0.49, 0.59, 0.74, 0.81	0.35, 0.47, 0.55, 0.60, 0.72

12	Shyonaka (Rt.)	0.07, 0.63	0.03, 0.09, 0.16, 0.59, 0.63, 0.73	0.12, 0.47, 0.59, 0.78, 0.85
13	Kantakari (Rt.)	-	0.08, 0.21, 0.25, 0.60	0.47
14	Marmelosin	0.55	0.55	0.55
15	Beta-sitosterol	-	-	0.48
16	Lupeol acetate	-	-	0.59

Shelf life Study of Dasmula Taila

Three batches of Dasmula taila formulation were prepared at Pharmacy department of CARI Kolkata and were kept in stability chamber at the Department of Chemistry for stability assessment and accelerated stability study. Understanding shelf life ensures that the prepared Dasmula Taila

maintain acceptable quality and safety standards.

Table 11 documents the comparative test result of Dasmula Taila kept in controlled environmental condition at temperature 38 °C and relative (RH) humidity 75% over a period of six months, with assessments at intervals of one month (01), three months (03), and six months (06).

Table 11: Comparative test result of *Dasmula Taila* kept in controlled environmental condition Temperature 38 °C and Relative (RH) Humidity 75% (Test reports after sample standing in Stability chamber)

Sl. N o.	Test parameters	Un it	0 day			1 st month			3 rd month			6 th month		
			B-I	B- II	B- III	B-I	B- II	B- III	B-I	B- II	B- III	B-I	B- II	B- III
1	Refractive index at 40 °C	-	1.4670	1.4665	1.4665	1.4691	1.4668	1.4701	1.4720	1.4705	1.4721	1.4735	1.4741	1.4742
2	Viscosity	mp a/s ec	86.91	82.73	83.51	89.95	87.37	85.25	96.21	91.29	92.61	101.5	95.96	97.82
3	Specific optical rotation		+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50	+0.50
4	Specific gravity	-	0.9196	0.9192	0.9198	0.9199	0.92	0.92	0.9199	0.92	0.9199	0.92	0.92	0.92
5	Saponification value	-	186.76	196.60	191.83	196.53	210.55	205.10	203.04	217.86	210.33	207.5	221.56	216.52
6	Unsaponifiable matter	-	1.20	1.22	1.25	1.21	1.24	1.26	1.23	1.25	1.28	1.25	1.29	1.30
7	Acid value	-	1.37	1.46	1.32	1.66	1.81	1.58	1.81	2.04	1.85	2.63	2.84	2.67
8	Iodine value	-	98.67	104.53	100.05	96.74	103.82	98.31	94.93	102.89	96.84	93.82	100.63	94.98
9	Peroxide value	-	NI L	NI L	NIL	NI L	NIL	NI L	NI L	NI L	NI L	NI L	NI L	NI L
10	Heavy metals													

	Cd	ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Pb	ppm	0.39	0.47	0.50	0.39	0.47	0.50	0.39	0.47	0.50	0.39	0.47	0.50
	As	ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Hg	ppm	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	Microbial load													
	Total microbial plate count	cfu / gm	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	Yeast and mould count	cfu / gm	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
12	Specific pathogens													
	E. coli	/ml	A	A	A	A	A	A	A	A	A	A	A	A
	Salmonella	/ml	A	A	A	A	A	A	A	A	A	A	A	A
	Pseudomonas aeruginosa	/ml	A	A	A	A	A	A	A	A	A	A	A	A
	S. aureus	/ml	A	A	A	A	A	A	A	A	A	A	A	A
13	Pesticide residue	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	Aflatoxins													
	B1	ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B2	ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	G1	ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	G2	ppb	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

The refractive index (RI) serves as an indicator of a sample's purity. It is commonly measured using refractometers in the pharmaceutical industry to ensure the quality of raw, intermediate, and final products. Typically, this measurement is conducted at the sodium D-line (NaD) wavelength of approximately 589 nm. The recorded values for batches I, II, and III are 1.4670, 1.4665, and 1.4665 respectively, suggesting consistency across the

batches with no significant variation. Viscosity is a fluid's resistance to flow or change shape at a specified rate. The values obtained in (Pa-sec) are 86.91, 82.73, 83.51 of batches I, II and III, respectively at 0 day and 101.5, 95.96, 97.82 (Pa-sec) of batches I, II and III at 6 months [Figure 3(a)] thus indicating that a slight increase viscosity of *Dasmula Taila* upon storage.

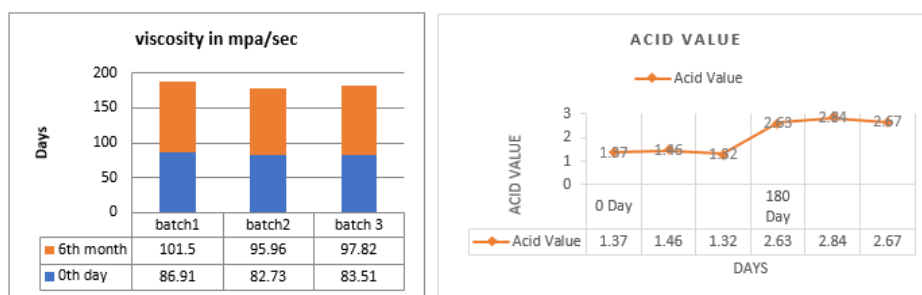


Figure 3. (a): Graph of Viscosity vs No. of days; (b): Graph of Acid value vs No. of days

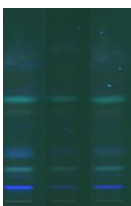
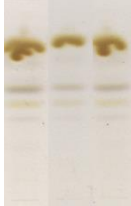
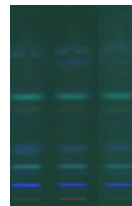
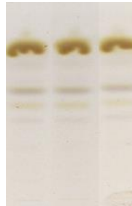
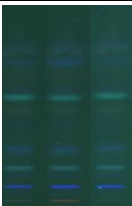
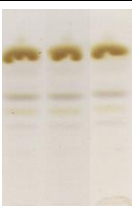
Specific rotation can also be used to test the purity of the material. The Specific Optical Rotation (SOR) is denoted by $[\alpha]$. SOR means examining the same at a specific temperature like - is constant at +0.50 for batches in the initial 0th day and at the 6th month. This formulation is stable. It can also be used to test the purity of the material. The specific gravity was measured at 40 °C. The values of batches did not show any significant changes in values of 0 day and 6th month. Thus, the formulation is stable. The saponification values of batches I, II and III at specific intervals of 0 day, 1st, 3rd, and 6th months showed an increase in Figure 3(b). The values were 186.76, 196.60, and 191.83 for batches I, II, and III on the 0th day to 207.55, 221.56 and 216.52 for batches I, II, and III at the end of six months, respectively. A greater saponification value indicates that the fatty acids present are, on average, shorter in chain length. There is no peroxide value, which suggests that the formulation is stable. Unsaponifiable matter is within the specified limit of 1.5, suggesting fit for topical usage. The highest acid value found at the 6th month suggests that prepared *Taila* could remain stable for up to 6 months, i.e. 180 days. The Iodine value typically reflects the oxidative resilience of fats, which is closely linked to the degree of unsaturation. This factor significantly influences the processing, longevity, and appropriate use of fat based products. The Iodine values of batches I, II and III at specific intervals

of 0 day, 1st, 3rd, and 6th months showed a decrease trajectory. The values were 98.67, 104.53 and 100.05 for batches I, II, and III on the 0th day to 93.82, 100.63 and 94.98 for batches I, II, and III at the end of six months, respectively.

High-Performance Thin Layer Chromatography Profile of *Dasmula Taila* (B-I, II, III)

For the analysis, 2 mL of each *Dasmula Taila* batch (B-I, II, III) was refluxed with 20 mL of Methanol for one hour. The mixture was filtered through filter paper, and the filtrate was reduced in volume for Thin Layer Chromatography (TLC). A TLC Silica Gel 60F₂₅₄ plate, supported on aluminium sheets, served as the stationary phase. The solvent mixture used was Toluene, Ethyl acetate, and Formic acid in a 7.5:2.5:0.4 (v/v) ratio. A 5 µL sample was applied as an 8 mm band, 15 mm from the plate's bottom. The plate, measuring 5x10 cm, was developed to 90 mm in a CAMAG Twin trough chamber under conditions of 25 °C and 42% humidity. Finally, 20% aqueous sulphuric acid was employed as the derivatising agent. Table 12 documents the comparative test result of HPTLC of *Dasmula Taila* kept in controlled environmental condition at temperature 38 °C and relative (RH) humidity 75% over a period of six months, with assessments at intervals of one month (01), three months (03), and six months (06).

Table 12: R_f values

Time		Photographs		
		254 nm	366 nm	White light
1st month Temperature 38 °C and Relative (RH) Humidity 75%		 I II III	 I II III	 I II III
R_f values	Batch-I	0.33, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.45, 0.48, 0.58, 0.71	0.47, 0.53, 0.61, 0.74
	Batch-II	0.32, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.47, 0.54, 0.64	0.45, 0.51, 0.57, 0.74
	Batch-III	0.32, 0.47, 0.74	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.48, 0.55, 0.65	0.45, 0.51, 0.58, 0.75
3rd month Temperature 38 °C and Relative (RH) Humidity 75%		 I II III	 I II III	 II III I
R_f values	Batch-I	0.33, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.45, 0.48, 0.58, 0.71	0.47, 0.53, 0.61, 0.74
	Batch-II	0.32, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.47, 0.54, 0.64	0.45, 0.51, 0.57, 0.74
	Batch-III	0.32, 0.47, 0.74	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.48, 0.55, 0.65	0.45, 0.51, 0.58, 0.75
6th month Temperature 38 °C and Relative (RH) Humidity 75%		 I II III	 I II III	 I II III

R_f values	Batch-I	0.33, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.45, 0.48, 0.58, 0.71	0.47, 0.53, 0.61, 0.74
	Batch-II	0.32, 0.47, 0.73	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.47, 0.54, 0.64	0.45, 0.51, 0.57, 0.74
	Batch-III	0.32, 0.47, 0.74	0.04, 0.06, 0.08, 0.15, 0.17, 0.20, 0.24, 0.28, 0.29, 0.32, 0.43, 0.48, 0.55, 0.65	0.45, 0.51, 0.58, 0.75

CONCLUSION

The findings of quality control parameters of *Dasmula Taila* i.e., refractive index (at 40 °C), specific gravity, acid value, iodine value, saponification value and unsaponifiable matter is incorporated in this study to establish the quality control benchmarks for the raw drug materials used, which will contribute to the development of the quality standards for the *Dasmula Taila* formulation. High-performance thin Layer Chromatography fingerprint profiling of *Dasmula Taila* formulation and its ingredients comply with the various reference standards. Accelerated shelf-life study of *Dasmula Taila* have been performed and reported first time. As no standard reference is available for this formulation, the present quality assessment report may be helpful for the preparation of the monograph and quality control data may be used as references which will setting the pharmacopeial standards for *Dasmula Taila* formulation. It may also help to assess the quality of this formulation for research and clinical purposes in future.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

This article includes all data generated or analyzed during this study.

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