



Phytochemical Screening and Standardization of Krishnadya Taila (A Polyherbal Ayurvedic Oil Based Formulation) through HPTLC

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ABSTRACT

Ancient literatures of Ayurveda speak about the importance of oil-based formulations. Krishnadya Taila is an ancient Ayurvedic polyherbal oil-based formulation. It is indicated in the various ocular disorders, refractive errors, headache, disorders of eyelids, in the form of Nasya (Administration of drugs by the route of nasal cavity). The present study was aimed to screen the organoleptic analysis, physico-chemical analysis, microbial limit test and phytochemical constituents through preliminary phytochemical tests of Krishnadya Taila and to standardize this herbal oil-based formulation through High Performance Thin Layer Chromatography fingerprinting. The preliminary phytochemical screening of the extract revealed the presence of bioactive compounds like Anthraquinone, Flavonoids, Alkaloid, Tannins and Polyphenols, Steroids and Terpenoids. The HPTLC fingerprint profile, obtained from this study, of the same herbal oil based formulation may be used for authenticity and quality analysis.

Keywords: Ayurveda, Krishnadya Taila, Polyherbal, oil-based formulation, Nasya, Phytochemical analysis, HPTLC fingerprinting, Standardization

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INTRODUCTION

Ancient literatures of Ayurveda include very well described knowledge of medicine practiced for thousands of years and has its own library of precious herbal formulas. These medicinal plants are rich sources of beneficial constituents and it is believed in Ayurveda that complex diseases can be treated with combination of medicinal plants rather than single drug [1]. Siddha Taila (medicated/processed oil) is prepared by protracted boiling of the *Sneha Dravya* (base oil) with prescribed *Drava Dravya* (liquid drug) and *Kalka Dravya* (drugs used as a fine paste) to dehydration or near dehydration. This process results in the transfer of some therapeutically active principles of the ingredients into the base oil. (Table 1)[2]. Thus, *Taila Paka Vidhi* (traditional method of Oil preparation) assures the enrichment of *Sneha dravya* with the active principles of the ingredients.

Krishnadya Taila is a polyherbal Ayurvedic oil formulation used for several *Netra roga* (eye ailments), *Timirroga* (refractive errors and Presbyopia) and *Shiroroga* (diseases of head) etc. [3]

Following are the ingredients used for the preparation of Krishnadya Taila: (Table 2)

- *Sneha Dravya*: Murcchita Tila Taila (processed oil from seeds of *Sesamum indicum* L.)
- *Drava Dravya*: Aja Paya (goat milk)
- *Kalka Dravya*: A paste obtained from the dried semi-powdered mixture of *Pippali* (*Piper Longum* Linn.), *Shunthi* (*Zingiber officinalis* Roxb.), *Vidanga* (*Embelia Ribes*), *Yasthimadhu* (*Glycyrrhiza Glabra*.) and *Saindhava Lavana*: Rock salt.

Quality control of Krishnadya Taila remains an unexplored issue. Thus, in the present work an attempt has been made to use some newer approaches for the standardization of Krishnadya Taila with following objectives:

- To develop Standardized Operating Procedure (SOP) for the preparation of Krishnadya Taila.
- To evaluate the Organoleptic, physico- chemical analysis, phytochemical identification and safety profile of Krishnadya Taila.

- To carry out chemical characterization of Krishnadya Taila on the basis of active principles of the ingredients using validated HPTLC method.

MATERIAL AND METHODS

Plant material

The ingredients (Table 1) were procured from the local market. The collected drugs were identified and authenticated at the teaching pharmacy of Department of Dravyaguna, Parul Institute of Ayurved, Limda, Waghodia, Vadodara.

Methodology of preparation of Krishnadya Taila [3]

Krishnadya Taila was prepared at GMP Certified- Parul Ayurved Pharmacy, Parul University, Limda, Vadodara, Gujarat.

The SOP for the preparation of KrishnadyaTaila involved following steps:

Preparation of Murchita Tila Taila [4] as per (Table 1): In the preparation of murchita Tila Taila (*tilatailamurchha*), five litres of TilTaila was taken and sixteenth part of it, Manjisthai.e (312.5 gm) was taken. Then *Musta*, *Haridra*, *Ushira*, *Lodhra*, *Ketaki*, (*Vata*) *Nyagrodha*, *KamalNaluka*, and *Triphala* (*Haritaki*, *Bibhitaki*, *Amalaki*) each drugs (78.125 grams) were taken as fourth part of Manjistha in (*Yavakut*) dried semi- powdered form. Water was taken as four part of Tilataila (20 litres approx.) The TilTaila was indirectly heated on a mild flame and these drugs in the mentioned amount were slowly added to it and stirred slowly and water was added slowly at intervals in it and was indirectly heated on a mild flame. The heating was continued till the mixture attained *Sneha Siddhi Lakshana*. [5] (completion test for chief desired characteristics) like, *Gandha Varna Rasotpatti* (desired smell, color and taste), *Shabdahinata* (no cracking sound), *Phenodgama* (appearance of froth) and *Vartivat Kalka* (rolling of paste of herbal drugs between fingers). The oil of amount 4.5 liters was obtained by filtration. The final Murchita Til Taila was obtained.

- Preparation of Kalka: Each Kalka Dravya (225 grams powder) was taken in a vessel and mixed, followed by addition of sufficient amount of water until a uniform paste was obtained.
- Preparation of KrishnadyaTaila :as per (Table 2), MurchitaTilaTaila(4.5 liters) was indirectly heated on a mild flame with, Saindhava Lavana (225 grams powder of Rock salt.) and Kalka obtained from Kalka Dravya, (a paste obtained from the semi-powdered mixture (*Yavkut*) of *Pippali* (*Piper longum*), *Shunthi* (*Zingiber officinale* roxb.), *Vidanga* (*Embllica Ribes*.), and *Yasthimadhu* (*Glycyrrhiza glabra*) of 225 grams each drug.) Mixture was stirred intermittently till it became slimy.

The heating was stopped and Aja Paya (Goat Milk of amount 18 litres) was added. The mixture was kept standing overnight. Next day, the heating was continued till the mixture attained *Sneha SiddhiLakshana* [5] (completion test for chief desired characteristics) like *Gandha Varna Rasotpatti* (desired smell, color and taste), *Shabdahinata* (no cracking sound), *Phenodgama* (appearance of froth) and *Vartivat Kalka* (rolling of paste of herbal drugs between fingers). Finally, the mixture was filtered when hot through muslin cloth and stored in small bottles until use.

Table 1: List of ingredients for the preparation of MurchitaTilaTaila (Tila Taila Murchha).

Sl. No.	Ingredients	Latin Name	Part Used	Quantity
1	<i>Manjishtha</i>	<i>Rubia cordifolia</i> Linn.	Stem	312.5 grams
2	<i>Ushira</i>	<i>Vetiveria zizanioids</i>	Root	78.125 grams
3	<i>Haridra</i>	<i>Curcuma Longa</i>	Rhizome	78.125 grams
4	(<i>Vata</i>) <i>Nyagrodha</i>	<i>Ficus benghalensis</i> L.	Root -Aerial	78.125 grams
5	<i>Ketaki</i>	<i>Pandanus fectonussoland</i>	Stem	78.125 grams
6	<i>Kamala Naluka</i>	<i>Nelumbo nucifera</i> Gaertn.	Stalk	78.125 grams
7	<i>Musta</i>	<i>Cyperus rotundus</i> L.	Rhizome	78.125 grams
8	<i>Lodhra</i>	<i>Symploeos racemose</i> Roxb.	Stem Bark	78.125 grams
9	<i>Haritaki</i>	<i>Terminalia chebula</i> Retz.	Fruit	78.125 grams
10	<i>Bibhitaki</i>	<i>Terminalia bellerica</i> Roxb.	Fruit	78.125 grams
11	<i>Amalaki</i>	<i>Embllica officinalis</i> (Gaertn.)	Fruit	78.125 grams
12	<i>Krishna TilaTaila</i>	<i>Sesamum indicum</i> L.	Sesame oil	5 liters
13	<i>Jala</i>	Water	-	20 liters

Table 2: List of ingredients for the preparation of KrishnadyaTaila

Sl. No.	Ingredients	Latin Name	Part Used	Quantity
1	Pippali	<i>Piper Longum Linn.</i>	Fruit	225 grams
2	Shunthi	<i>Zingiber officinale roxb</i>	Rhizome	225 grams
3	Vidanga	<i>Emblica Ribes</i>	Fruit	225 grams
4	Yasthimadhu	<i>Glycyrrhiza Glabra</i>	Root	225 grams
5	Saindhava Lavana	Rock salt	Salt	225 grams
6	Tila Taila (Murcchita)	<i>Sesamum indicum L.</i>	Processed Sesame oil	4.5 liters
7	Aja Paya	Goat Milk	Goat Milk	18 liters

Phytochemical analysis

Preliminary phytochemical screening and phytochemical studies through HPTLC were carried out at Vasu Research Centre, Makarpura, Vadodara-390010, Gujarat, India as per the standard procedures.

Preliminary phytochemical tests [6, 7]

The preliminary phytochemical screening was performed according to the standard procedure. The procedures are as follows:

Test for alkaloids

Wagner's test: About 1ml of extract and 1ml of Wagner's reagent (dilute iodine solution) are added and mixed. Formation of reddish-brown precipitates indicates the presence of alkaloids.

Dragendroff's Test

To a few milligrams of extract dissolved in alcohol, a few drops of acetic acid and dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

Mayer's Test

To a few milligrams of extract dissolved in acetic acid, a few drops of mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

Hager's Test

To a few milligrams of extract dissolved in acetic acid, 3 ml of hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Test for carbohydrates**Molisch's Test**

To the extract, 1 ml of α -naphthol solution and conc. sulphuric acid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

Fehling's Test

A few milligrams of extract were mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's Test

To 5 ml of Benedict's reagent, a few milligrams of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates

Test for steroids**Libermann Burchard Test**

To the extract dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of con. H_2SO_4 were added along the sides of the test tube. Appearance of bluish green color indicates the presence of steroids.

Salkowski Test

The extract was dissolved in chloroform and equal volume of con. H_2SO_4 was added. Formation of bluish red to cheery red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for Saponins

To a few milligrams of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for Tannin

To the extract a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

Test for Flavonoids

Shinoda's test: To the extract in alcohol, a few magnesium turnings and few drops of conc. H_2SO_4 were added and heated on a water bath. Formation of red red to pink colour indicates the presence of flavonoids.

Test for Phenol

To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue black indicates the presence of phenol.

Test for Coumarins

To the extract in alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for Triterpenoids

The extract was warmed with tiny bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for Carboxylic Acid

Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few milligrams of the sample were mixed with water and acetone. Turbidity indicates the presence of resin.

Test for quinine

A few milligrams of alcohol extract were treated with 0.5% of sodium hydroxide. Deep colouration like pink, purple or red indicates the presence of quinine.

High Performance Thin Layer Chromatography [8, 9]**Preparation of Test Solution**

0.1 ml of sample is taken in a test tube and diluted it with 1 ml of Hexane, it is mixed well. The test solution thus obtained was used for HPTLC fingerprinting.

Preparation of Spray reagent [Anisaldehyde – sulphuric acid reagent]

0.5 mL Anisaldehyde is mixed with 10 mL Glacial acetic acid, followed by 85 mL Methanol and 5 mL Sulphuric acid (98 %). 3.0 µl of the above extract were applied on a pre-coated Silica gel 60 F₂₅₄ on aluminum sheets to a band width of 10 mm using CAMAG Linomat 5 TLC applicator. The plate was developed in Petroleum ether: Diethyl ether: Acetic acid (9 : 1 : 0.1 v/v/v). The developed plates were visualized in short UV 254, 366, and then derivatized with Anisaldehyde Sulphuric acid reagent and scanned under UV 254nm, 366 nm and 540 nm. R_f and densitometric scan were recorded.

RESULTS AND DISCUSSION**Organoleptic and Physico-chemical Analysis**

Organoleptic and Physico-chemical characters of “KrishnadyaTaila” are illustrated in (Table 3). The description provides as Dark yellow to brown coloured Taila (Oil) having characteristic odour. The obtained drug has Specific gravity as 0.9160 and Refractive index as 1.475. The drug having Viscosity by Ostwald as 37.1 cP, Acid value as 6.03 and Peroxide value as 13.04.

Table 3: Organoleptic and Physico-Chemical Analysis of KrishnadyaTaila

Sr.No	Parameters	Results
1	Description	Dark yellow to Brown coloured oil
2	Odour	Characteristic
3	Specific gravity	0.9160
4	Viscosity by Ostwald	37.1 cP
5	Refractive index	1.475
6	Acid value	6.03
7	Peroxide value	13.04

Table 4 :Phytochemical constituents of KrishnadyaTaila

Sr. No.	Parameters	Results
1	Alkaloid	+
2	Starch	-
3	Tannins and Polyphenols	+
4	Saponins	-
5	Flavonoids	+++
6	Carbohydrates	-
7	Proteins	-
8	Steroids	++
9	Terpenoids	++
10	Anthraquinone	+

Key word: “+, ++, +++” indicates Present in increasing intensity and “-” indicates Absent.

Table 5 : Microbial Limit Test of KrishnadyaTaila

Sr. No.	Parameters	Result
1	Total Yeast & Mould Count (TYMC)	Absent
2	<i>Staphylococcus aureus</i>	Absent
3	<i>Salmonella sp.</i>	Absent
4	<i>Pseudomonas aeruginosa</i>	Absent
5	<i>Escherichia coli</i>	Absent

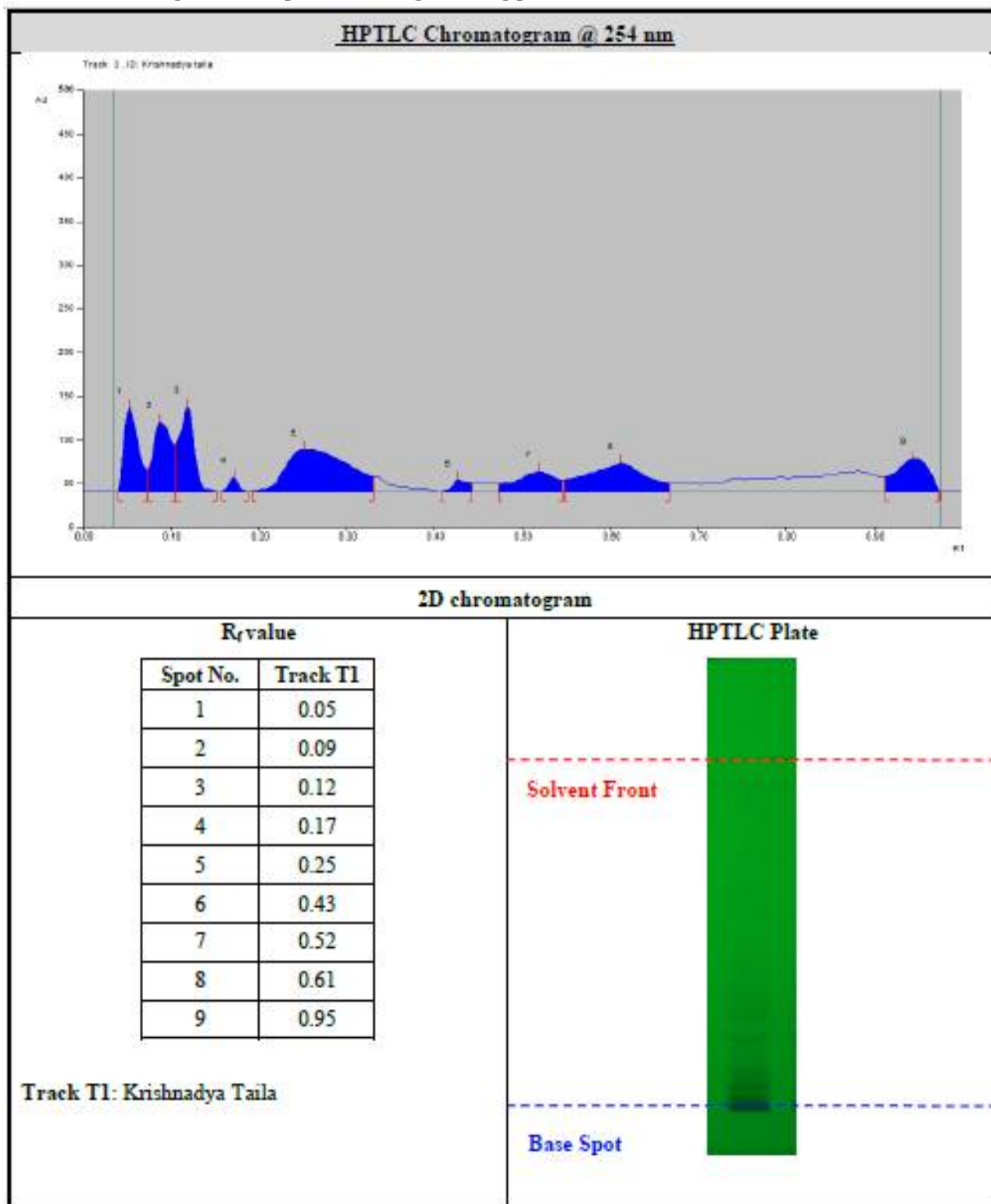
Fig.1. HPTLC plate showing banding pattern and R_f Values at 254 nm

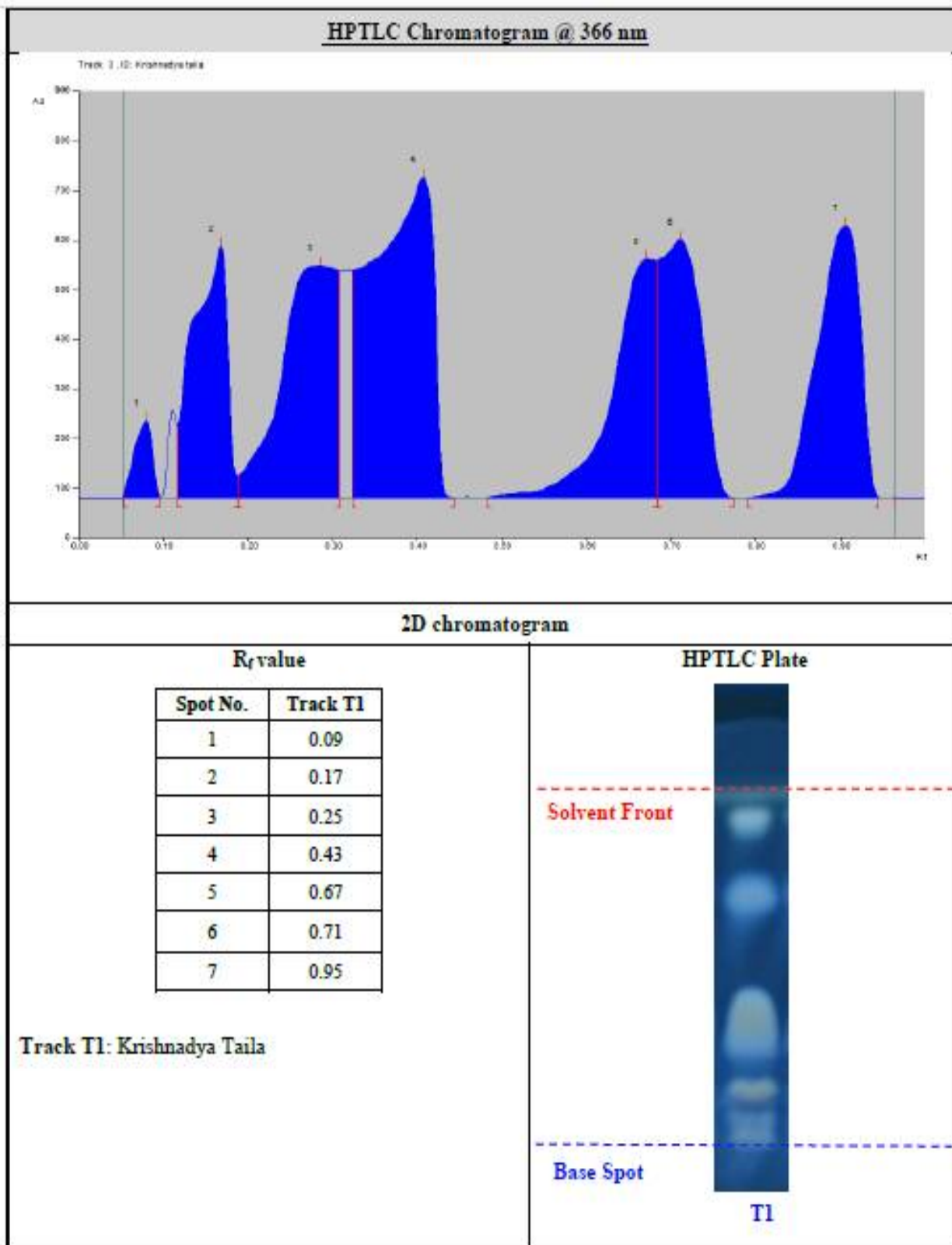
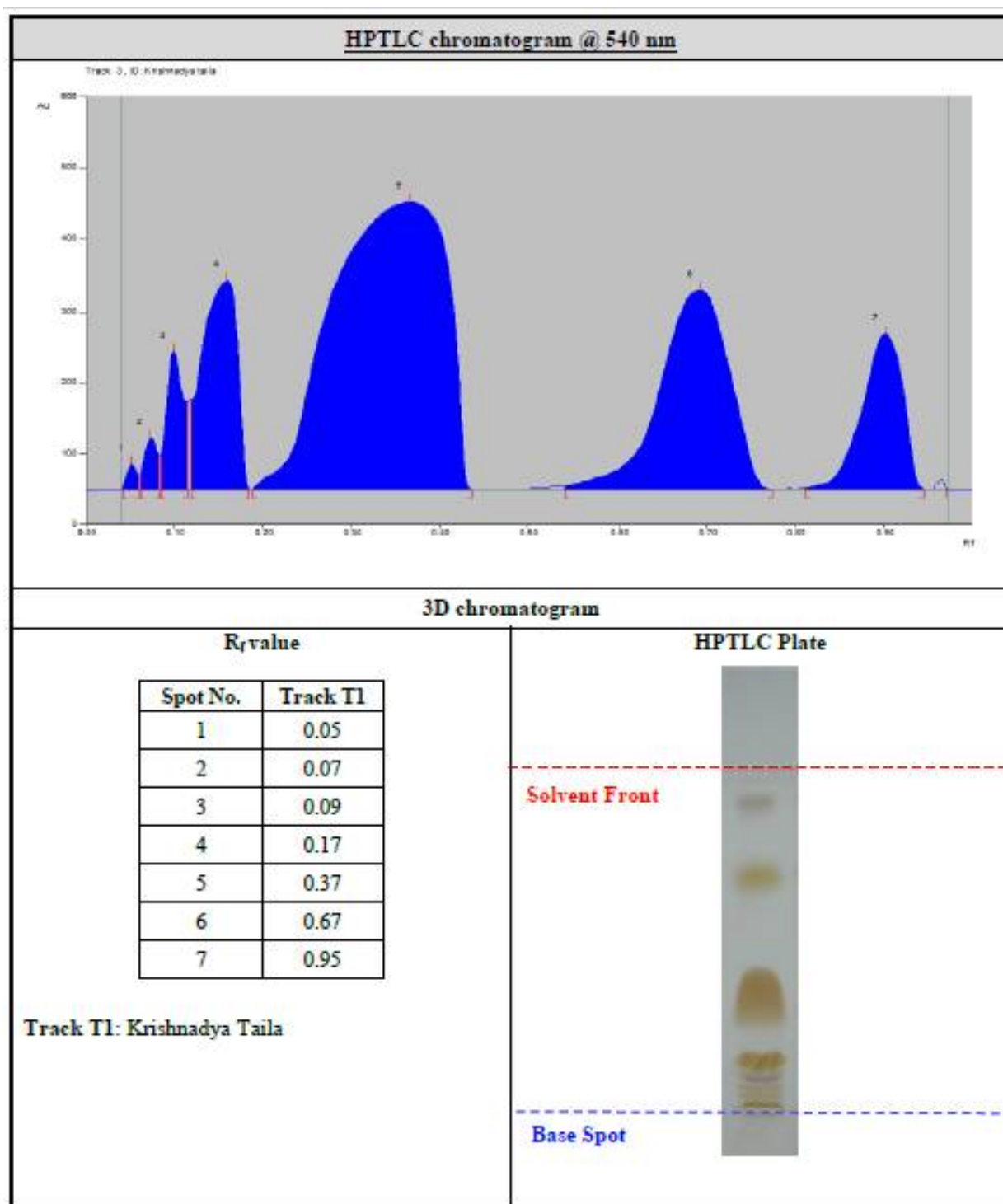
Fig. 2. HPTLC plate showing banding pattern and R_f Values at 366 nm

Fig. 3. HPTLC plate showing banding pattern and R_f Values at 540 nm

Preliminary Phytochemical Tests

The phytochemical screening results showed the presence of Alkaloids, Tannins and polyphenols, Flavonoids, Steroids, Terpenoids and Anthraquinone, in the extract of drugs of “KrishnadyaTaila” (Table 4). Most of the identified phytochemical compounds have been reported to have various biological activities like Flavonoids induce the expression of antioxidant proteins that can help protect cells from oxidative stress, thus potentially providing long-term protection of the eye. Flavonoids can function as potent and effective neuroprotective agents for retinal ganglion cells[10]. Flavonoids may have a positive effect on eye conditions associated with visual dysfunction. Some studies reported that flavonoids are bioavailable to the eye in quantities that can affect signal transduction mechanisms and influence enzymatic functions within the eye. Flavonoids also have a favorable effect on conditions associated with

visual impairment [11]. Flavonoids are capable of acting on various mechanisms or etiological factors responsible for the development of different sight threatening ocular diseases. Local administration of these agents may yield much higher and effective concentrations of the parent bioflavonoids in the ocular tissues [12]. Terpenoids is source of potential geroprotectors, Antioxidant effects of volatile terpenes and terpenoids have also been demonstrated in brain cells in vitro as well as *in vivo* [13]. Terpenoids can effectively influence the mechanisms of aging and age-related diseases. It has anti-inflammatory properties and antioxidant activity which has effects on stress-resistance on eyes [14]. A range of pharmacological properties of anthraquinones has been reported, which include anti-oxidant, anti-ulcer, anti-inflammatory, anti-cancer, neuroprotective, anti-aging, lung protective, and hepatoprotective properties [15]. Several pharmacological properties are attributed to alkaloids as a result of their antibacterial, antiproliferative, and antioxidant effects. It also possesses anti-inflammatory, antioxidant properties and have benefit in eye irritations [16].

Tannins have Effects on oxidative damage of ocular lens. The protective effect of tannin against oxidative damage in lens is already examined and proved. Polyphenols may have a direct role on receptors, like in the case of Rhodopsin [17].

High Performance Thin Layer Chromatography

HPTLC photo documentation of "KrishnadyaTaila" (Fig -1) showed Nine, Seven and Seven spots under 254 nm, 366 nm and 540 nm after derivatization respectively. Spot with Rf value 0.05 and 0.25, 0.43, 0.67, were commonly detected in any two detection methods. Spot with Rf value 0.09, 0.17 and 0.95 were commonly detected in all three detection methods. All the three methods gave optimum separation of different bands and hence all of them may be used as HPTLC fingerprint pattern to identify the composition of the mixture (Fig. 1,2,3). Densitometric scan at 254 nm revealed 3 high peak and 6 peaks corresponding to 9 different compounds in the ethanol extract, compounds with Rf value 0.05, 0.09, 0.17 were the high peaks (Fig- 1). At 366 nm there were Six high peak and one low peak corresponding to 7 different compounds in the ethanol extract, with Rf value 0.17, 0.25, 0.43, 0.67, 0.71, 0.95, being the major peak detected (Fig- 2). At 540 nm there were seven peaks and Five high peaks, with Rf value 0.09, 0.17, 0.37, 0.67 and 0.95 being the major peaks detected (Fig- 3).

Microbial Limit Test

While evaluating the microbial limit test, the total yeast and mould count, *Staphylococcus aureus*, *Salmonella sp*, *Pseudomonas aeruginosa* and *Escherichia coli* were found absent. (Table 5).

CONCLUSION

Preliminary phytochemical tests of the extract of KrishnadyaTaila showed the presence of alkaloids, tannins and polyphenols, flavonoids, steroids, terpenoids and anthraquinone, which are reportedly bioactive in nature and may add up to the therapeutic effect of this polyherbal drug. HPTLC fingerprint profile of the same polyherbal formulation may be used for authentication and quality control. So, it can be concluded that these parameters can be used for the evaluation of Krishnadya Taila. The present study can serve as the reference for the future works on Krishnadya Taila.

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