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PHYTOCHEMICAL SCREENING AND STANDARDIZATION OF PUSHPA VARTI (A POLYHERBAL AYURVEDIC OPHTHALMIC FORMULATION) THROUGH HPTLC

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ABSTRACT

Many of the ophthalmic herbal formulations are described in classical literatures of Ayurveda- the traditional Indian system of medicine, as local applications in the form of *vartis* (Pills), *anjana* (Collyrium), and eye drops etc. These formulations are very effective in the management of eye diseases. *Pushpavarti* is a herbal ophthalmic formulation. It is used in the various eye aliments like refractive errors, presbiopia, pterygium, cysts, scar, subconjunctival hemorrhage and any growth in conjunctiva's in the form of anjana (Collyrium). Pushpavarti constitutes four drugs (dried flower of *Sesamum indicum*, and *Jasminum grandiflorum* and dried fruit of *piper longum* and *piper nigrum*). The present study was aimed to screen the organoleptic analysis, physico-chemical analysis, microbial test limit and phytochemical constituents through preliminary phytochemical tests of Pushpa Varti and to standardize this poly herbal mixture through High Performance Thin Layer Chromatography fingerprinting. The preliminary phytochemical screening of the extract revealed the presence of bioactive compounds like alkaloid, Tannins and polyphenols, saponins, flavonoids, steroids and Terpenoids. The HPTLC

fingerprint profile, obtained from this study, of the same herbal formulation may be used for authenticity and quality analysis.

Keywords: Ayurveda, Bioactive compounds, Pushpa Varti, Polyherbal formulation, Phytochemical analyses, HPTLC fingerprinting, standardization

INTRODUCTION

Ayurveda consists of well-organized knowledge of medicine being practiced for thousands of years and it possess its own library of valuable herbomineral formulations. 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]. Majority of the remedies arebased on plants and plants products along with minerals as well as animals origin. These medicine systems also described some formulations as local application in the form of *vartis*, *anjana*, eye drops etc.These generally improve the resistance, immunity, strengthen the organ or system and alleviate the ailments [2]. Tila *(sesamum indicum)* [3], Pippali*(piper longum)* [4], *Jati (Jasminum grandiflorum)* [5] and Maricha*(piper nigrum)*[6] are such medicinal plants having various medicinal properties and used to treat various diseases. In this study Extract of Tila, Pippali, *Jati* and Maricha*i.e.* proportional mixture of useful parts **(Table 1)** of these four herbs is used for preliminary phytochemical screening and standardization through High Performance Thin Layer Chromatography (HPTLC). In the present studyan effort has been made to determine the phytochemical constituents of "*Pushpavarti*" as well as to standardize this particular formulation which will provide another useful resource for future acceptance at global level.

MATERIALS AND METHODS

Plant material

The ingredients **(Table 1)** were procured from the local market and herbal gardens of Parul Institute of Ayurved. 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 Pushpa Varti [7]

Table 1: Ingredients (Plant materials)

Sl. No.	Ingredients	Latin Name	Part Used	Quantity
1	*TilaPushpa*	*Sesamumindicum*	Flower	5 part
2	*Pippali*	*Piper longum*	Fruit (inner part)	4 part
3	*Jati*	*Jasminum grandiflorum*	Flower	3 part
4	*Maricha*	*Piper nigrum*	Fruit	1 part

Pushpavarti was prepared at GMP Certified-Parul Ayurved Pharmacy, Parul University, Limda, Vadodara, Gujarat. The Flowers of Tila *(sesamum indicum)*, outer layer of fruits of Pippali (*piper longum*) were peeled and grains(inner part of Fruit) of Pippali (*piper longum*) *were collected*, flowers of Jati (*Jasminum grandiflorum*), fruit of Maricha (*piper nigrum*) were collected. These ingredients were taken in proportions (5:4:3:1) in sequence and dried. After drying these drugs weremade into fine powdered form and mixed well.They were mixed with water (with bhavanadravyas of the above drugs). Then these were made into a nice fine paste, rolled into small pills (Varti) and dried. This obtained drug "Pushpa Varti" was stored in a closed vessel and stored as accelerated stability study under (Temperature 40^{+}/-2 ^{0} C and 75^{+}/-5% RH) at Vasu Research Centre, Makarpura, Vadodara-390010, for furthur use.

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 [8, 9]

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 choloro form, 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.H2SO4 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 tin 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 was mixed with water and acetone. Turbidity indicates the presence of resin.

Test for quinine

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

High Performance Thin Layer Chromatography [10, 11]

Preparation of Test Solution

2.5 g of sample is weighed in an Iodine Flask and to it 50 ml of Methanol is added. Vortex the iodine flask for 1 hour to dissolve the sample. Then the sample is filtered through Whatman Filter Paper which further filtered with syringe filter and the filterate of the sample is taken. The filterate thus obtainedwas 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 %). 10.0µl of the above extract were applied on a pre-coated Silica gel 60 F_{254} on aluminum sheets to a band width of 10 mm using CAMAGLinomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Acetic acid (7: 2:1 v/v/v). The developed plates were visualized in short UV 254, 366, and then derivatised with Anisaldehyde Sulphuric acid reagent and scanned under UV 254nm, 366nm and 540nm. Rf and densitometric scan were recorded.

Table 2: Organoleptic and Physico-Chemical Analysis of Pushpa Varti

Sr. No.	Parameters	Results
1	Description	Brown coloured
2	Odour	Characteristic
3	Average weight	124.5 mg
4	Touch	Smooth
5	Texture	Hard
6	Disintegration time	59 min
7	pH	6.78

Table 3: Phytochemical constituents of PushpaVarti

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	Anthraquinine	-
Key word: "+, ++, +++" indicates Present in increasing intensity and " - " indicates Absent.		

Table 4: Microbial Limit Test

Sr. No.	Parameters	Result
1	Total Yeast & Mould Count	Absent
2	*Staphylococcus aureus*	Absent
3	*Salmonella sp.*	Absent
4	*Pseudomonas aeruginosa*	Absent
5	*Escherichia coli*	Absent

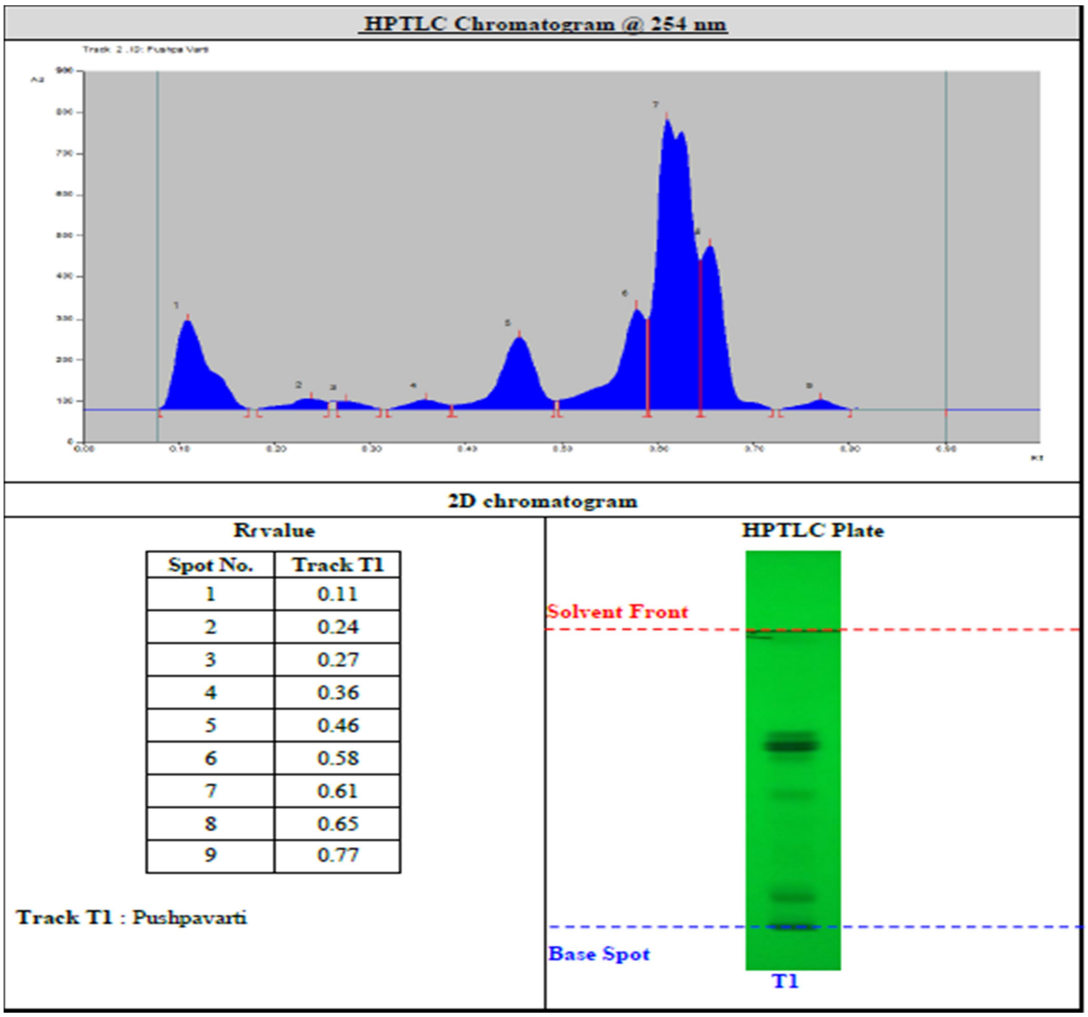

Fig. 1: HPTLC plate showing banding pattern and Rf Values at 254 nm

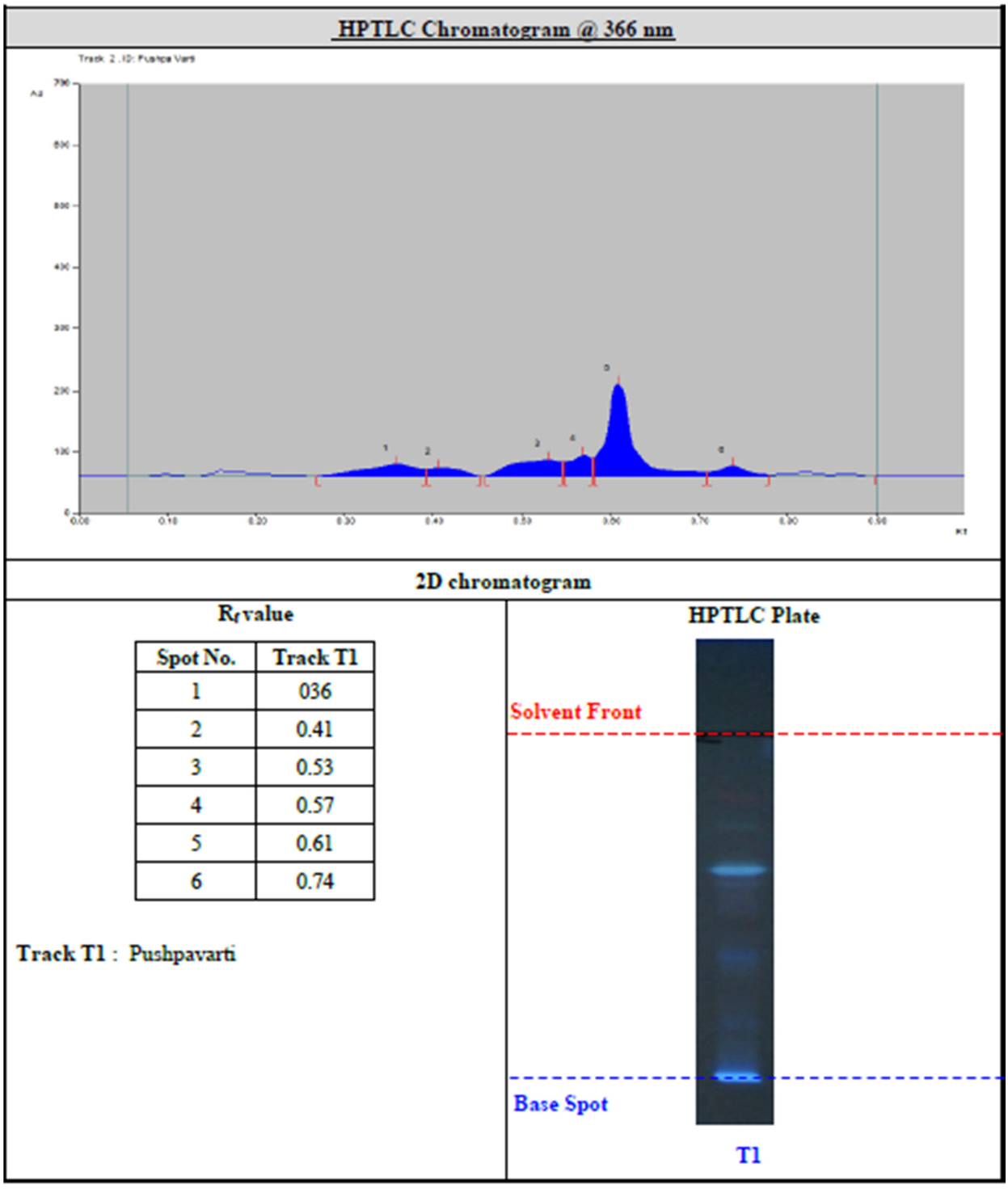

Fig. 2: HPTLC plate showing banding pattern and Rf Values at 366 n

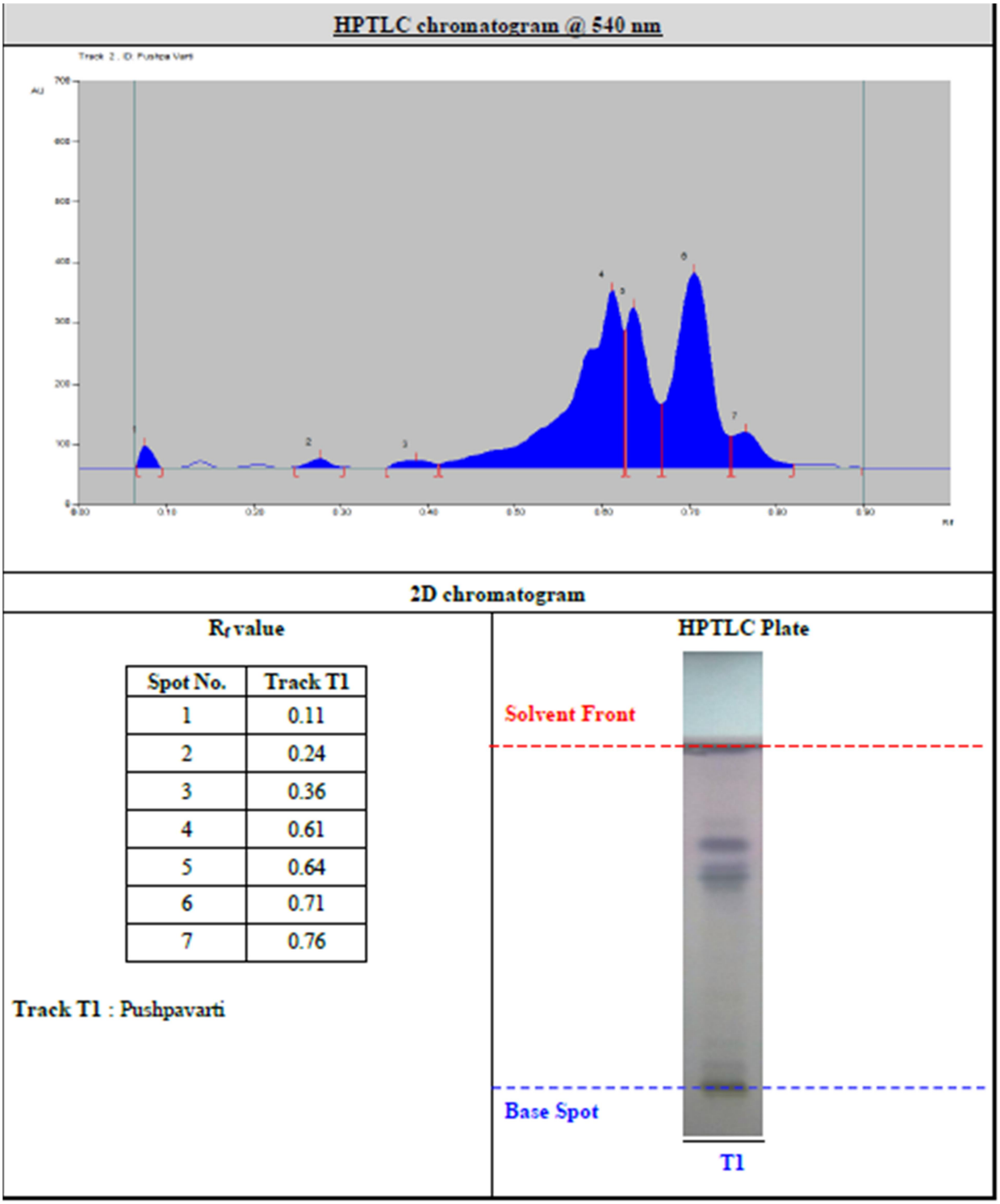

Fig. 3: HPTLC plate showing banding pattern and Rf Values at 540 nm

RESULTS AND DISCUSSION

Organoleptic and Physico-chemical Analysis:

Organoleptic and Physico-chemical characters of *Pushpavarti* are illustrated in **(Table 2)**. The description provides as brown coloured Varti (Pills) having hard texture and smooth in touch. The obtained drug have characteristic odour with average weight as 124.5 mg and disintegration time of 59 minutes with pH value as 6.78.

Preliminary Phytochemical Tests:

The phytochemical screening results showed the presence of alkaloids, Tannins and polyphenols, saponins, flavonoids , steroids and Terpenoids in the extract of drugs of "Pushpavarti" **(Table 3)**. Most of the identified phytochemical compounds have been reported to have various biological activities *viz*. the alkaloids possess anti-inflammatory, antioxidant properties; Antispasmodic ,benefit in eye irritations, decongestant and diuretic [12], Polyphenols possess antioxidant properties, useful in retinal degenerative diseases of eyes, improve the stability, expression, regeneration and folding of rhodopsin, It improve the integration of the receptor into the cell membrane while acting against oxidative stress at the same time. The neuroprotective effect on the retina observed in flavonoids may be associated with its modulating effects on specific cellular pathways related to antioxidant mechanisms , polyphenols may have a direct role on receptors, like in the case of Rhodopsin[13]. Saponins are natural antioxidant and anti-inflammatory drugs, It has hemolytic properties [14,15]. Flavonoids improve microvascular functions , reduce intraocular hypertension and increase ocular blood flow, Flavonoids within the fruits and vegetables may operate in the prevention of both age-related and diabetic cataract via multiple mechanisms including lowering oxidative stress, Flavonoids showed some protection against cataract formation, Flavonoids acts as antioxidant, anti-inflammatory and anti-angiogenic mechanisms to stabilize collagen and to improve microvascular integrity, may aid in restoring proper RPE (retinal pigment epithelium) functions. Flavonoids demonstrate antioxidant properties and diminish oxidative damage to the lens of the eye. It can function in the eye to stabilize collagen and improve microvascular integrity, both important to slowing retinopathies and development of glaucoma. Flavonoids have anti-angiogenic. Diets high in flavonoid-rich fruits and vegetables have been shown to lower risk of vision disorders, including diabetic retinopathy, glaucoma,

and cataracts. Flavonoids are bioavailable to the eye in quantities that can affect signal transduction mechanisms and thus could affect gene regulation and enzyme function within the eyes[16]. Steroids are anti-inflammatory agents and has better chance of visual improvement with early diagnosis of visual loss[17]. Terpenoidsis source of potential geroprotectors, that can effectively influence the mechanisms of aging and age-related diseases.Ithasanti-inflammatory properties andantioxidant activity which haseffects on stress-resistance on eyes. [18]

High Performance Thin Layer Chromatography:

HPTLC photo documentation of *Pushpavarti* **(Fig -1)** showed Nine, Six and Seven spots under 254 nm, 366 nm and 540 nm after derivatization respectively. Spot with Rf value 0.11 and 0.24, were commonly detected in any two detection methods. Spot with Rf value 0.36 and 0.61, 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 5 high peak and 4 peaks corresponding to 9 different compounds in the ethanol extract, compounds with Rf value 0.11, 0.46, 0.58, 0.61 and 0.65 were the high peaks (Fig- 2). At 366 nm there was one high peak, with Rf value 0.61, being the major peak detected (Fig- 2). At 540 nm there were seven peaks and three high peaks, with Rf value 0.61, 0.64 and 0.71 being the major peaks detected **(Fig- 3)**.

Microbial Limit Test

While evaluating the microbial limit test, the total yeast and mould count, *Staphylococcus aureus*, *Salmonellasp*, *Pseudomonas aeruginosa* and *Escherichia coli*were found absent. **(Table 4)**

CONCLUSION

Preliminary phytochemical tests of the extract of *Pushpavart* showed the presence of alkaloids, tannins and polyphenols, saponins, flavonoids, steroids and terpenoids. Phenols, 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 *Pushpavarti.* The present study can serve as the reference for the future works on *Pushpavarti.*

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