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"INGREDIENTS IDENTIFICATION, PHYSICO-CHEMICAL AND HPTLC EVALUATION OF *VAJIGANDHADI TAILA* – A POLYHERBAL FORMULATION"

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ABSTRACT

Vajigandhadi Taila (VT) is a classically well-known medicated oil for *Gridhrasi* disease. To standardize the VT medicament with pharmacognostical, physico-chemical, Chromatographical profiling according to ASU guidelines. The *Taila* are prepared with respective pharmacognostically identified ingredients *Vajigandha/Ashwagandha* (*Withania somnifera* Linn.), *Bala* (*Sida cordifolia* Linn.), *Bilva* (*Aegle marmelos* Corr.), *Brihati* (*Solanum indicum* Linn.), *Kantakari* (*Solanum surrattense* Burm.f.), *Prishaniparni* (*Uraria picta* Desv.), *Shalaparni* (*Desmodium gangeticum* DC.), *Gokshura* (*Tribulus terrestris* Linn.), *Agnimantha* (*Premna mucronata* Roxb.), *Shyonaka* (*Oroxyllum indicum* Vent), *Patala* (*Stereospermum suaveoleus* DC), *Gambhari* (*Gmelina arborea* Linn.), and along with base *Eranda Taila* (castor oil) with standard operating procedure. The VT was subjected to standardize by physico-chemical and chromatographical parameters. Pharmacognostical identified features of coarse powder are systemically explained and physico-chemical finding of VT formulation such as Acid value, Refractive index, Iodine value, Saponification value and Specific Gravity respectively was found 10.57, 1.472, 91.09, 168.97043 and 0.9577. The chromatographic authentication of VT was done under pre-chromatographic derivatization technique. Microscopic characteristics like the pitted vessels, oil globules, rhomboidal crystal, starch grains, prismatic crystals, shows that ingredients presence in VT formulation. The findings from this study will provide systemic evaluation and also serve as a master document to control the quality of VT formulation.

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INTRODUCTION

Gridhrasi comes under 80 types of *Nanathmaja Vatavyadhi* [1]. It is characterised by severe pain starting from low back region and radiating down towards the foot. This is a common entity encountered in a clinical practice. Sciatica is a condition where there is distribution of pain along the course of sciatica nerve, which the pain radiates from low back to buttock and leg [2]. It is commonly known as being caused by lumber disc herniation. Sciatica of contemporary medical science is closely equivalent to *Gridhrasi* by its similarity in the course of pain from low back radiating down through the posterior part of leg. *Vajigandhadi Taila* [3] is described in *Yogaratnakara*, *Vatavyadhi Adikarna* with specified indication in *Gridhrasi.* In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to provide scientific basis for the traditional herbal medicines that are claimed to possess effect in Sciatica. The first step for scientifically based research is to provide quality standardization of drug. With this background the present study was undertaken to establish the authenticity of all the ingredients of *Vajighandhadi taila* and incidence of constituents as suggested through pharmacognostical, physico-chemical and HPTLC evaluation of *Vajighandhadi taila*.

AIMS AND OBJECTIVES

1. To identify dry samples of ingredients of *Vajigandhadi Taila* Macroscopically and Microscopically.
2. To preliminary analyze the *Vajigandhadi Taila* by using different physico-chemical parameters.
3. To develop the HPTLC profile of *Vajigandhadi Taila.*

MATERIALS & METHOD

The study was done in following steps:

1. Collection of the raw drugs
2. Microscopical evaluation of powdered raw drugs
3. Preparation method of *Vajigandhadi Taila*
4. Organoleptic study of prepared drug
5. Physico-chemical analysis
6. HPTLC (High Performance Thin Layer Chromatography) evaluation

Collection of Raw Drug

All the raw drugs of *Vajigandhadi Taila* were obtained from Pharmacy, Gujarat Ayurved University, Jamnagar, India and all these were identified and authenticated in Pharmacognosy Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda (IPGT & RA), Gujarat Ayurved University, Jamnagar, India.
Ingredients of *Vajigandhadi Taila* are concise at [Table 1].

Table No. 1: Ingredients of *Vajigandhadi Taila.*

Sr. No.	Drugs	Botanical name	Part used
1	*Vajigandha (Ashwagandha)*	*Withania somnifera* Linn.	Root
2	*Bala*	*Sida cordifolia* Linn.	Whole plant
3	*Bilva*	*Aegle marmelos* Corr.	Bark
4	*Agnimantha*	*Premna mucronata* Roxb.	Stem
5	*Shyonaka*	*Oroxyllum indicum* Vent	Bark
6	*Patala*	*Stereospermum suaveoleus* DC	Bark
7	*Gambhari*	*Gmelina arborea* Linn.	Bark
8	*Brihati*	*Solanum indicum* Linn.	Whole plant
9	*Kantakari*	*Solanum surrattense* Burm. f.	Whole plant
10	*Shalaparni*	*Desmodium gangeticum* DC.	Whole plant
11	*Prishniparni*	*Uraria picta* Desv.	Whole plant
12	*Gokshura*	*Tribulus terrestris* Linn.	Whole plant
13	*Eranda taila*	*Ricinus communis*	Seed Oil

Microscopical evaluation of powdered raw drugs of *Vajigandhadi Taila*

In certain limits it is possible to analyse the finished products for the pharmacognosy i.e. Compound formulations like *Choorna* (powder), *Vati* (tablet), *Kalka* (paste) etc. It was difficult to analyse the *Taila* to find out the cellular level of raw drugs. In this study as *Vajigandhadi Taila* was made from *Kalka* (paste) & *Kwatha* (decoction) of *Ashwagandha, Bala, Bilva & Dashmoola* thus raw drugs powders individually were studied separately with and without staining. The micro pictures were taken under Carl zeis microscope attached with camera [4].

Preparation method of *Vajigandhadi Taila*

Vajigandhadi Taila was prepared in RS and BK *(Rasashastra* and *Bhaishajya Kalpana)* department, Institute for Post Graduate Teaching and Research in Ayurved, Gujarat *Ayurved* University, Jamnagar, India. All identified drugs were washed and dried properly. Since *Kwatha* (decoction) and *Kalka* (paste) *dravyas* (drugs) are not mentioned separately by *Yogratanakar* so the same drugs in the equal proportion i.e. *Aswagandha, Bala, Bilva* and *Dasamoolam* (10 drugs as 1part) are taken for *Kwatha* and *Kalka* preparation. *kwatha* was prepared by adding 8 times water in equal amount of *Aswagandha, Bala, Bilva* and *Dasamoolam* and then boiled in low flare to decrease it to 1/4th of total water [5]. The *Kalka*, *Taila* and *Kwatha* for preparation of *Vajigandhadi Taila* were taken in the proportion 1: 4: 16 as per classical reference [6]. After preparation of *kalka* and *kwatha*, pure *Eranda Taila* (Castor Oil) was measured and poured into a brass vessel with thick base on medium flare. The *Kwatha* and *Kalka* was also poured into the vessel and the mixture was boiled in medium flame with continuous stirring and monitoring of *Paka*. The boiling was stopped and the oil was sieved by using a washed and dried white filter cloth when *Madhyama Paka* [7] was attained.

Organoleptic study of prepared drug

Organoleptic study of prepared *Vajigandhadi Taila* are endangered for various sensory characteristics like odour, colour etc. were carefully distinguished down. [Table No. 3]

Physico-chemical analysis

Physico-chemical analysis of *Vajigandhadi Taila* was done by using various standard physico-chemical parameters such as Acid value [8], Refractive Index value, Saponification value, Iodine value [9], and Specific gravity [10] at Pharmaceutical chemistry laboratory, IPGT and RA, Jamnagar, India. Physico-chemical analysis were carried out by following standard procedure mentioned in API (Ayurvedic Pharmacopeia of India).

HPTLC (High Performance Thin Layer Chromatography) evaluation [11]

Sample was prepared by diluting 1 ml *Vajigandhadi Taila* with 2 ml Hexane and it was used for spotting. Prepared sample of *Vajigandhadi Taila* was spotted on pre-coated silica gel aluminium plate as 6mm bands by means of a CAMAG Linomat V sample applicator fitted with a 100µL Hamilton syringe. Then alcoholic KOH was applied on same spotted area and plate was heated at 110^{o}c on TLC plate heater for 10 minutes. Hexane: Diethyl Ether (7:3) was used for *Vajigandhadi Taila* as a mobile phase. The development time was 30 minutes. After development, Densitometry scanning was performed with a CAMAG TLC scanner III in reflectance absorbance mode at 254 nm and 366 nm under control of Win CATS software (V1.3.4 CAMAG).Then the plate was dipped in 10% H_2So_4 followed by heating and then visualized in day light. The R_f values and colour of resolved spots were noted.

OBSERVATIONS & RESULTS

Microscopic Characters

Powder microscopy characters of individual herbal drugs of *Vajigandhadi Taila* were observed as below [Table No. 2] and microphotographs are placed at respective plate. [Plate No. 1]

Table No. 2.

Sr. No.	Drugs	Identified Microscopic Characters	Sr. No.	Drugs	Identified Microscopic Characters
1	*Vajigandha (Ashwagandha)*	Border Pitted Vessels, Starch Grains, Cork cells	7	*Gambhari*	Border pitted vessels, Stone cells
2	*Bala*	Oilglouble, Romboidal Crystal, Stellete Trichome	8	*Brihati*	Pitted Vessels with Starch Grains, Stone cells, Simple trichome
3	*Bilva*	Fibre with crystal, Scleroid, Starch simple &compound	9	*Kantakari*	Multi branch Trichome with Fibers, Stone cells
4	*Agnimantha*	Rhomboidal crystal, Cork cells	10	*Shalaparni*	Pitted & Annular vessel, Trichome, Epidermal cells
5	*Shyonaka*	Cigar shaped crystals, Pitted stone cells	11	*Prishniparni*	Prismatic crystal & cork cells, Spiral vessels
6	*Patala*	Crystal fibres, Prismatic crystals	12	*Gokshura*	Epidermal cells, Prismatic crystals

Organoleptic Characters:

Organoleptic characters of prepared *Vajigandhadi Taila* carefully observed and distinguished as below. [Table No. 3]

Table No. 3.

Parameter Studied	Observations
Color	Opaque Brown
Odour	Slightly Bitter Aromatic
Consistency	Slightly thick & Single thread

Physico-chemical results:

Physico-chemical findings of prepared *Vajigandhadi Taila* are given in below table. [Table No. 4]

Table No. 4.

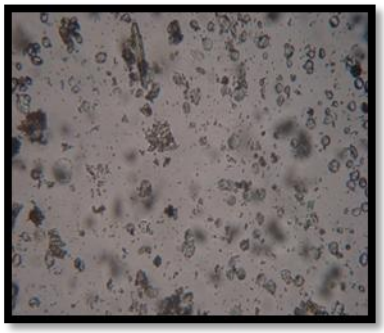
Parameter studied	Results
Acid value	10.57
Refractive Index	1.472
Iodine value	91.09
Saponification Value	168.97043
Specific Gravity	0.9577

Table No. 5: R_f values.

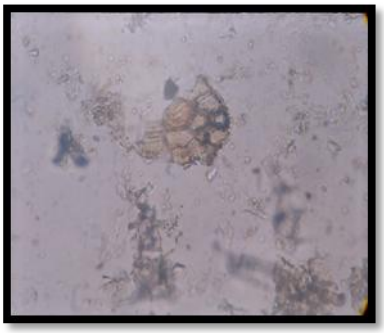
Sample	Visualize under short UV (254 nm)		Visualize under long UV (366 nm)	
Vajigandhadi Taila	No. of spots	Rf value	No. of spots	Rf value
	5	0.03, 0.14, 0.36, 0.45, 0.87	4	0.03, 0.36, 0.43, 0.93

Plate No. 1: Microphotographs of *Vajigandhadi Taila* Ingredients-

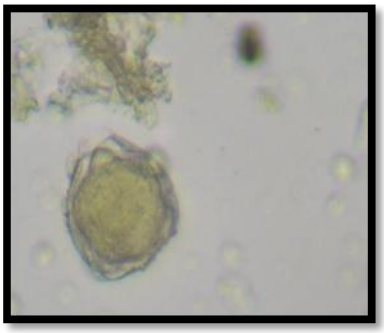
Border Pitted Vessels (*Ashwagandha*)



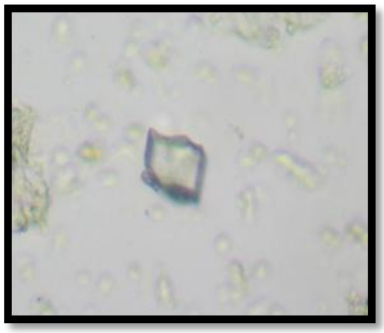
Starch Grains (*Ashwagandha*)



Cork cells (*Ashwagandha*)



Oil globule *(BALA)*

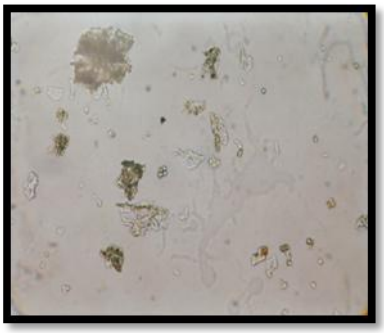


Rhomboidal crystal *(BALA)*

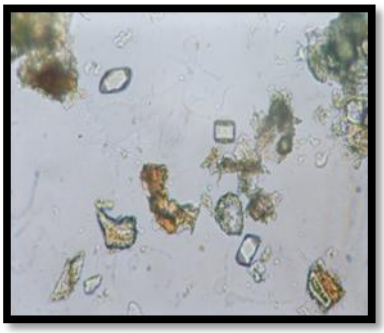
Stellate trichome *(BALA)*

Fibre with crystal *(Bilwa)*

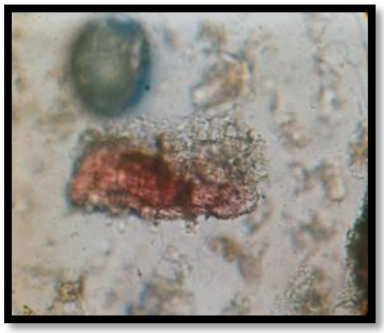
Scleroid *(Bilwa)*



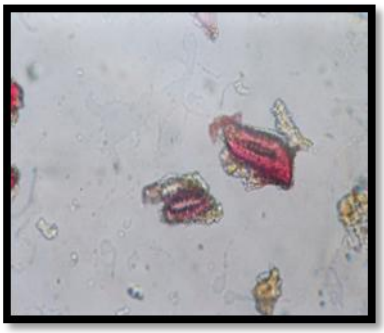
Starch simple & compound *(Bilwa)*



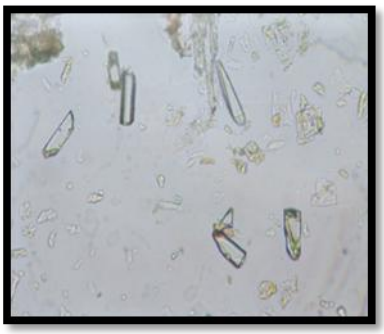
Rhomboidal crystal (*Agnimantha)*



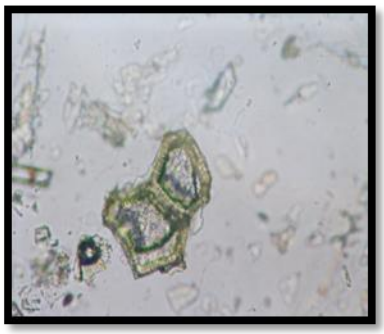
Cork cells (*Agnimantha)*



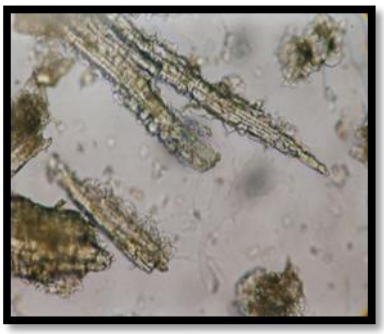
Stone cells (*Agnimantha)*



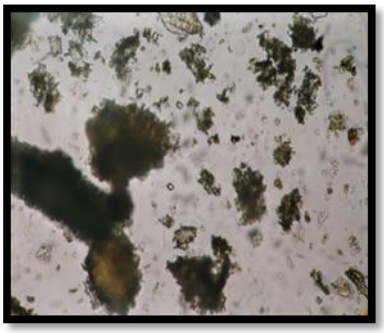
Cigar shaped crystal *(Syonaka)*



Pitted stone cell *(Syonaka)*

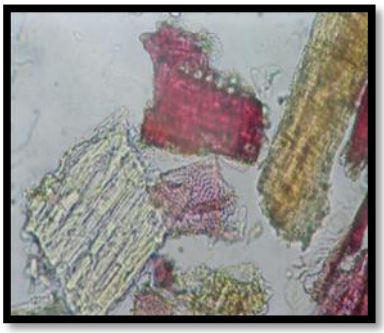


Crystal fibres *(Patala)*

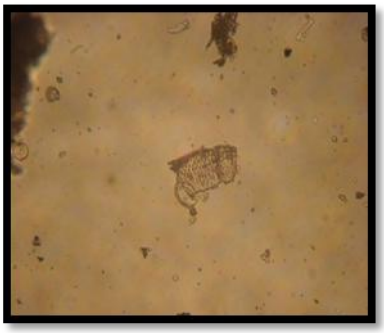


Prismatic crystal *(Patala)*

Border pitted vessels *(Gambhari)*



Stone cells *(Gambhari)*



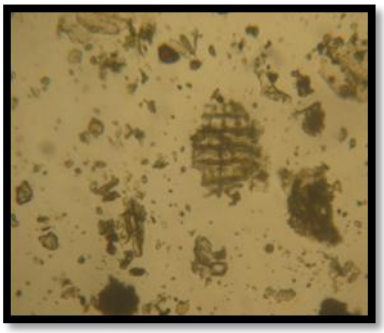
Pitted vessels with starch grains *(Brihati)*

Stone cells *(Brihati)*

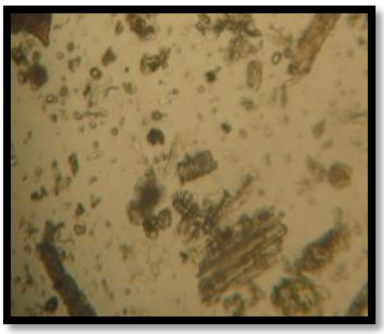
Simple trichome *(Brihati)*

Multi branch trichome with fibres *(Kantakari)*

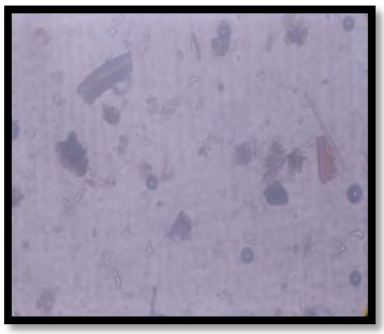
Stone cells *(Kantakari)*



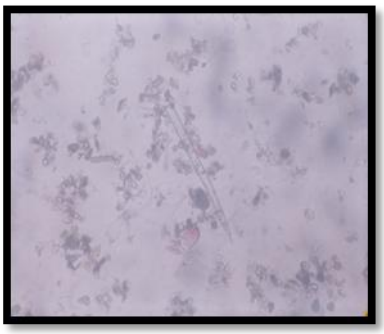
Prismatic crystal & cork cells *(Prishniparni)*



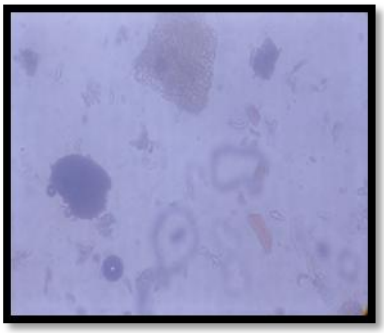
Spiral vessels *(Prishniparni)*



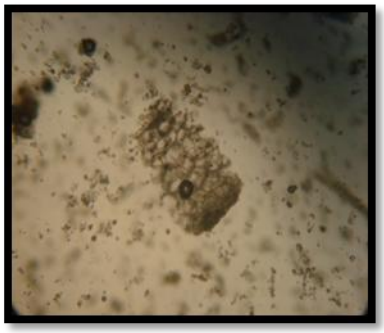
Pitted & Annular vessels *(Shalaparni)*



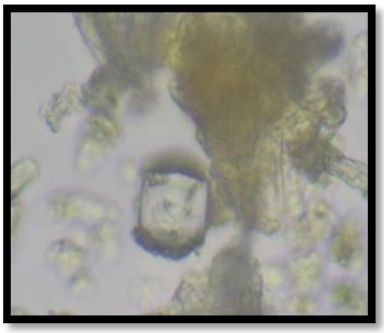
Trichome *(Shalaparni)*



Epidermal cells *(Shalaparni)*

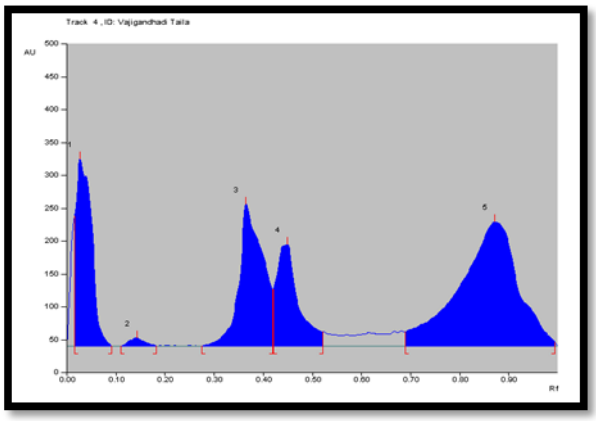


Epidermal cells *(Gokshura)*

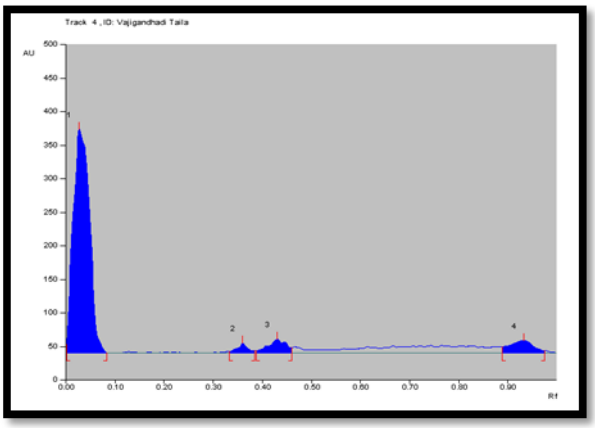


Prismatic crystals *(Gokshura)*

Plate No. 2: Densitogram at 254 nm and 366nm.

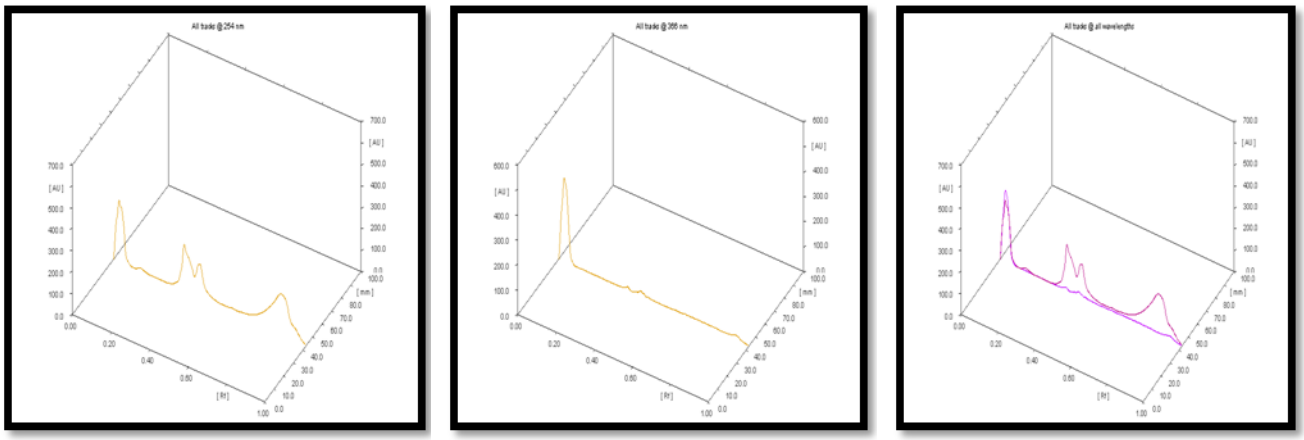


At 254nm



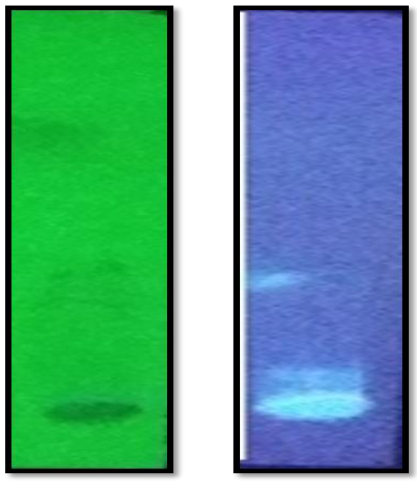
At 366nm

Plate No. 3: Three dimensional (3D) Densitogram At 254nm, 366nm & Comparison at 254nm & 366nm.



At 254nm **At 366nm** **Comparison at 254nm & 366nm**

Plate-4: HPTLC finger prints at 254nm and 366nm



At 254nm **At 366nm**

DISCUSSION

The Pharmacognostical study exposes authentication of individual raw drugs of *Vajigandhadi Taila* and it is cross verified in *Ayurvedic* Pharmacopeia of India (API) [12]. The pitted vessels, oil globules, rhomboidal crystal, starch grains, prismatic crystals, fibres etc. were observed in ingredients. Quality control parameters like specific gravity, saponification value are standard for any fat or oil. Similarly, when oil-fats become rancid, triglycerides are converted into fatty acids and glycerol [13] causing an increase in acid value, iodine value and refractive index suggestive of oxidation [14]. The oxidation levels of vegetable oils are important quality criteria in food chemistry because oxidation increases their toxicity by the formation of products such as hydroperoxides, aldehydes, ketones, etc.[15] All physico-chemical parameters; acid value, iodine value, saponification value, specific gravity, refractive index analyzed were almost near to the reference range as identified for *Vajigandhadi Taila* [16]. In this study *Vajigandhadi Taila* is well separated compact symmetrical bands in favour of chromophore sensitive component (Sterol, phytosterol, stigmasterol etc.) indirectly due to prechromatographic derivatization of oil sample directly. By visualization under short UV there 5 spots at 0.03, 0.14, 0.36, 0.45, 0.87 R_f while under long UV exposure 4 spots at 0.03, 0.36, 0.43, 0.93 R_f (Table 5/Plate 2 & 3). Component represent by the R_f 0.03 and 0.36 were common in both light exposure.

CONCLUSION

The pharmacognostical evolution proved that ingredients existing in the *Vajigandhadi Taila* (VT). It is concluded that the formulation meets maximum qualitative standards based on physico-chemical parameters. The separation pattern of VT is documented with help of prechromatographic derivative method in context of R_f & densitogram. The findings from this study will provide systemic evaluation and also serve as a master document to control the quality of *Vajigandhadi Taila* formulation. The study results may be used as the reference standard in further research undertakings of its kind.

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Conflict of interest: NiL

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