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RESEARCH ARTICLE

PHARMACOGNOSTICAL AND PHYSICO-CHEMICAL EVALUATION OF KRISHNADI TAILA: A POLY HERBAL COMPOUND IN THE MANAGEMENT OF ASTIGMATISM

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ABSTRACT

Background: The WHO has grouped Astigmatism and uncorrected refractive error among the leading causes(18 %) of blindness and vision impairment in the world. KRISHNADI TAILA (KT) is a poly herbal Ayurvedic formulation mentioned in Yogratnakara Netrarogadhikara. Though it is an important drug until date no pharmacognostical work is found on it. **Objective:** The present study was aimed at setting a standard pharmacognostical and pharmaceutical profile of KT. **Materials and Methods:** Study included preparation of KT following all SOPs using raw drugs, which were previously authenticated. Later, KT was subjected to pharmacognostical, physicochemical and high performance thin-layer chromatography (HPTLC) analysis as per standard protocols. **Results:** The final observations were recorded. Pharmacognostical findings matched with that of individual raw drugs with no major change in the microscopic structure of the raw drugs during preparation of Ghana Vati. HPTLC gave the fingerprint of the formulation with ten and eight spots on short and long UV, respectively. **Conclusion:** This shows presence of certain constituents in the powder and is helpful for the easy separation of these constituents.

Keywords: Krishnadi Taila, Astigmatism, Phyto-Constituents, HPTLC.

INTRODUCTION

The importance of vision and its organ i.e eye needs no description because for a blind person day and night are same and this world is of no use inspite he has a lot of wealth.[1] In Astigmatism - a refractive error where in the patient along with the blurred vision perceives the object as distorted one, which nearly resembles the vataja type of timira. On the aetiopathological grounds refraction which differes in different meridian due to altered curvatures of refractive media i.e cornea, lens at different meridians.[2] As far as the treatment of ocular diseases is concerned; it is clearly mentioned that before doing Netra Tarpana (satiating of eye with medicated / nonmedicated ghee) Shiro-Shodhana is to be carried out first. So, keeping this point in view, KT were selected for Shiro-Shodhana (Nasya).[3] According to Acharya *Charaka*, "A drug, that is not understood perfectly is comparable to poison, weapons, fire and the thunderbolt; while, the perfectly understood drug is comparable to ambrosia; and drug or diet article that reverses or break the *Samprapti(pathogenesis)* is ideal. It is often the total effect of all the ingredients in the formula rather than the action of individual drugs that plays a vital role in therapeutics. So pharmacognostical study of the contents of Krishnadi Taila was done for its identification and authentification and physio - chemical evaluation for ideality of prepared drug.

MATERIALS AND METHODS

Collection, Identification and Authentication of raw drugs:

Dried specimens of useful parts of herbal ingredients viz., Krishna, Vidanga, Madhuyashtika, Sindhujanma, Vishvaushadha and were procured from the Department of Pharmacy, IPGT&RA, GAU, Jamnagar. *Tila taila* was procured from *Khadi Gramodyoga Pharmacy*, Jamnagar. Cow milk was procured from the local Bhavani Milk Centre, Patel Colony, Jamnagar. **(Table-1)** All the herbal drugs were confirmed to be authentic and of good quality by the Pharmacognosy Laboratory, IPGT & RA, GAU, Jamnagar. The test drug KT was prepared as per classical reference and physicochemical and qualitative analysis of the final product was carried out in our laboratory of IPGT & RA, GAU, Jamnagar.

Table 1: Ingredients of Krishnadi Taila

Sr. No.	Drug Name	Botanical Name	Part Used	Parts
1	Krishna	Piper longum Linn.	Fruit	1 Part
2	Vidang	Embelia ribes Burm.	Fruit	1 Part
3	Madhuyashtik	Glycyrrhiza glabra Linn.	Root	1 Part
4	Sindhujanm	Rock Salt	Whole	1 Part
5	Vishvaushadh	Zingiber officinale Roxb.	Rhizome	1 Part
6	Aja Paya	Goat milk	-	80 part
7	Tila Taila	Sesamum indicum L.	Sesame oil	20 part

Organoleptic parameters:

Table 2: Macroscopic Features

Drug Name	Part Used	Nature	Colour	Taste	Odour
Krishna	Fruit	Fine Powder	Reddish Grey	Pungent	Characteristics
Vidanga	Fruit	Fine Powder	Brownish	Astringent	Characteristics
Madhuyashtika	Root	Fine Powder	Brownish	Astringent	Characteristics
Sindhujanma	Whole	Fine Powder	Whitish Grey	Sour	Characteristics
Vishvaushadha	Rhizome	Fine Powder	Greenish white	Bitter	Characteristics
Aja Paya	-	Liquid	Yellowish white	Bitter, Pungent and Astringent	Pungent
Tila Taila	Sesame oil	Liquid	Greenish black	Astringent	Charecteristics

Krishnadi Taila was prepared out of all the ingredients as per the Taila preparation method. Coarse Powder of all the herbal drugs was subjected for organoleptic characters which are enlisted in **Table 2**. The prepared test drug Krishnadi Taila (Fig 15) is in a liquid form, oily in touch, yellowish-black in colour with Characteristic odor and astringent in taste.

Powder Microscopy:

Powder microscopy of each raw drug was made with powder of dried samples by studying under the Carl Zeiss Microscope before and after staining with Phluroglucinol and concentrated HCl to study the characters of the drug **(Table 3)**. The microphotographs were taken and attached with the microscope.

[A] Krishna (Pippali):

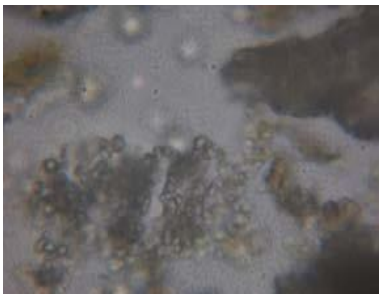

Fig 1: Starch grains without hilum

Fig 2: Stone cells

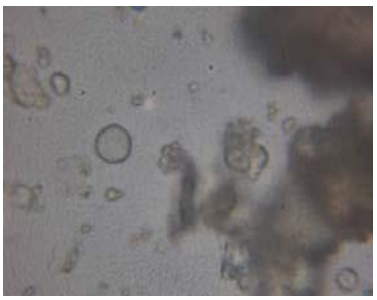

Fig 3: Oil globules

[B] Vidanga:

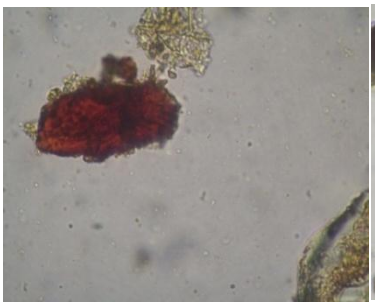

Fig 4: Fibre with wide lumen

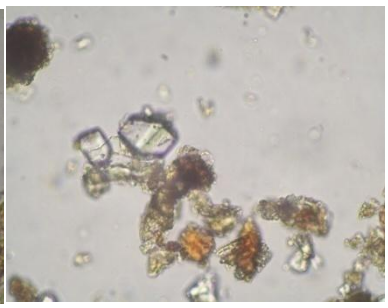

Fig 5: Fragment of annular vessels

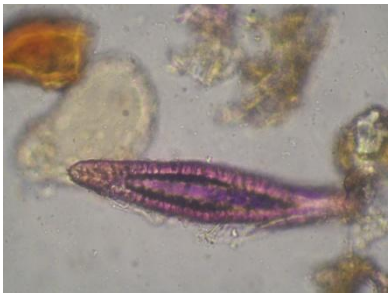

Fig 6: Pitted stone cells with wide lumen

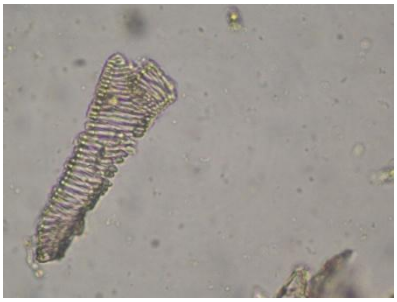

Fig 7: Rhomboid Crystles

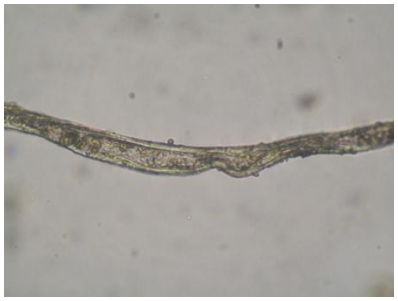

Fig 8: Tanin

[C] Madhuyashtika:

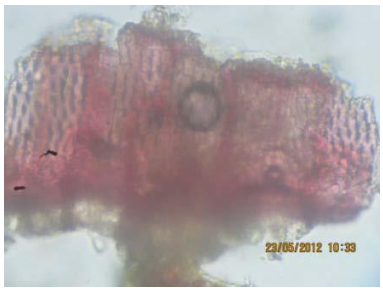

Fig 9: Pitted Vessels

Fig 10: Simple Fibre

Fig 11: Crystal Fibre

[D] Vishvaushadha (Shunthi):

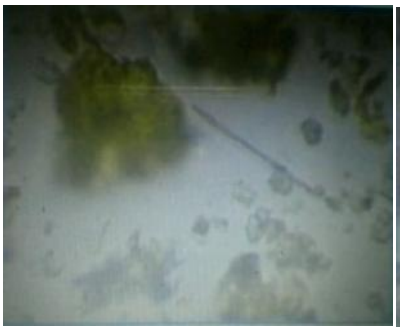

Fig 12: Oleoresin

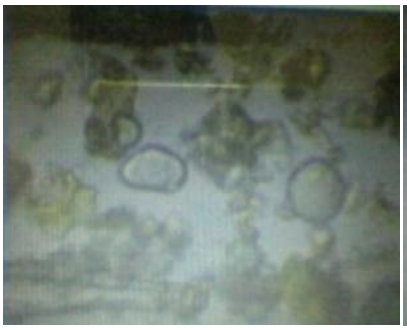

Fig 13: Simple starch grains

Fig 14: Annular Scalariform vessels

Table 3: Results of Powder Microscopy

(++ Seen/Present, -- Not Seen/Absent)

Features Identified	Krishna	Vidanga	Madhuyashtika	Vishvaushadha
Starch grains without hilum	++	--	--	--
Fibre with wide lumen	--	++	--	--
Fragment of annular vessels	--	++	--	--
Rhomboid Crystles		++	--	--
Tanin	--	--	--	--
Pitted Stone cells	++	++	--	--
Pitted Vessels	--	--	++	--
Simple Fibre	--	--	++	--
Crystal Fibre	--	--	++	--
Oleoresin	--	--	--	++
Simple starch grain	--	--	--	++
Annular Scalariform vessels	--	--	--	++
Oil globules	++	--	--	--

Preparation of the Drug:

Preparation of Coarse Powder:

The herbal drugs enlisted from 1 to 5 **(Table 1)** were washed, dried and a stipulated quantity made into coarse powder and divided into two parts. Out of this major part ($3/4^{th}$) was used for *kwatha* preparation and smaller portion ($1/4^{th}$) used for preparing *Kalka*.

Preparation of *Kwatha* (Decoction):

Decoction was prepared based on *Sharangadhara Samhitas* general rule by mixing above said coarse powder of the drugs with water in the ratio of 1:8, which was there after heated at medium temperature, till it reduced to one fourth of its original quantity and filtered multiple fold thoroughly to avoid fine particles in the final filterate (decoction).

Preparation of *Kalka* (Paste):

The second part of the raw drug was taken and mixed with required quantity of water to convert into paste form.

Taila Paka Vidhi: This was carried out as per the method prescribed in the classics [4]. Initially *Tila Taila* kept in iron vessel was subjected for *Moorchana*. After completion of *moorchana*, temperature raised to 70^0 C. At this moment *Kalka* was added with continues stirring. To this decoction, cow milk was added with continues stirring. After proper mixing of

all the ingredients, temperature was maintained between 65^0 C to 100^0 C, heating was carried out for two days, on second day after attaining *Madhyama Paka Taila sidda lakshanas*, final product of *Krishnadi Taila* was collected, filtered thoroughly and stored in a iron vessel for one month before subjecting for usage.

Fig 15: Krishnadi Taila

Physicochemical Parameters and Qualitative Analysis:

Krishnadi Taila was analyzed by using qualitative and quantitative parameters at Pharmaceutical Chemistry Laboratory, IPGT&RA, GAU, Jamnagar. All Physico-chemical parameters such as Acid value, Iodine Value, Saponification Value, Refractive Index and specific Gravit ywere determined. **(Table 4)**

Table 4: Physico-Chemical Parameters Of Krishnadi Taila

Sr. No.	Analytical parameters	Results
1	Acid value	2.2645
2	Iodine value	80.70 %
3	Saponification value	144.57
4	R.I.	1.489
5	Specific gravity	0.9677

(R. I.: Refractive Index)

The water and methanol extract of the sample was analyzed qualitatively for different functional groups. In qualitative method, presence of flavonoids, Phytosterons, Isoflavones tannins, saponin, alkaloids, glycosides and carbohydrates were assessed **(Table 5).** [5]

Table 5: Qualitative Analysis of Krishnadi Taila

Functional Group	Sample
Flavonoids	Positive
Tannins	Positive
Alkaloids	Positive
Saponin	Positive
Carbohydrates	Positive
Glycosides	Positive

TLC and HPTLC were carried out after preparing an appropriate solvent system with methanolic extract of *Krishnadi Taila.*[6]

HPTLC were performed for the normal phase separation of components of methanol extracts of *KT*. HPTLC study of the methanol extract was also carried out by using the solvent system of Hexane : Diethyl ether : Acetic Acid 6.5 : 2.5 : 1.0 ratios. After completion of HPTLC; post chromatographic derivatization was done with vanilline sulphuric acid.[7]

RESULT & DISCUSSION

RESULTS

The initial purpose of the study was to confirm the authenticity of the drugs used in the preparation of KT. For that, detailed organoleptic evaluation was carried out for the course powder of all the herbal ingredients of KT.

The powder microscopy of the Pippali revealed presence of Starch grains without helium, Stone cells, Oil globules. (Fig 1-3) *Vidanga* showed Fragment of Fibre with wide lumen, Fragment of annular vessels, Pitted stone cells with wide lumen, Rhomboid Crystles and Tanin. (Fig 4-8) *Madhuyashtika* showed Pitted Vessels, Simple Fibre, Crystal Fibre. (Fig 9-11)

Shunthi showed Oleoresin, Simple starch grains, Annular Scalariform vessels. (Fig 12-14) The diagnostic features obtained by powder microscopy were compared with the standards mentioned in Ayurvedic Pharmacopeia of India (API). The details of Pharmacognostical study are enlisted in the **Table 3.** [8]

Physicochemical parameters and Qualitative Test:

Qualitative tests indicated presence of alkaloids, tannins, glycosides, saponin, flavonoids, protein, carbohydrates and steroid. Details are shown in the **Table 5**.

DISCUSSION

Pharmacognosy study helps in authentication of commonly used drugs through morphological, histological and Physico-chemical parameters. This can prevent the accidental misuse of drugs and adulteration to a greater extent. In the present study the formulation consist of five herbal ingredients which were proved to be genuine by assessing the Pharmacognostical parameters. The presence of flavonoids, tannins, alkaloids, saponin, isoflavones, carbohydrates and glycosides etc., are the commonest features seen in all the ingredients.

Evaluation of Physico-chemical parameters and qualitative analysis helps to assess the quality and identify the presence of specific ingredients in a formulation and application of chromatographic techniques which aid in recognition of number of ingredients and also to assess the purity by comparing with the standard ones. Refractive Index Value 1.489, Specific Gravity

Table 6: Results of Hptlc of Krishnadi Taila

Extract	Solvent system	Wavelengths	Spots	Rf value
Methanol	Hexane : Diethyl ether : Acetic Acid (6.5 : 2.5 : 1.0)	254 nm	10	0.01, 0.13, 0.20, 0.33, 0.39, 0.47, 0.56, 0.64, 0.81, 0.89
		366 nm	08	0.01,0.27, 0.33,0.38, 0.73,0.81, 0.88,0.94

0.9677, Acid Value 2.2645, Saponification Value 144.57, Iodine Value 80.70%, all the parameters were within the standard limit. HPTLC is the most common form of chromatographic method used by Ayurvedic research workers to detect the number of compounds present in a product. It also helps to determine the purity of the sample. Identification of a compound is also possible by comparing it with the Rf value of a known compound. Here for the purpose of conducting HPTLC of KT tracks was made having the sample methanol extract. After careful analysis and discussion with experts the mobile phase was fixed to be Hexane : Diethyl ether : Acetic Acid 6.5 : 2.5 : 1.0 in ratio V/V. The sample tracks and mobile phase remained the same for all the experiments related to HPTLC. The spots produced by HPTLC were observed in short UV(254nm) and long UV(366nm) and Rf value was calculated.Track showed 10 spots under 254nm with Rf 0.01, 0.13,0.20, 0.33,0.39, 0.47,0.56, 0.64,0.81, 0.89 and 08 spots were seen under 366 nm with Rf 0.01,0.27,0.33,0.38,0.73,0.81,0.88,0.94 values. Details are noted in the **Table 6** and Fig 16 and 17.

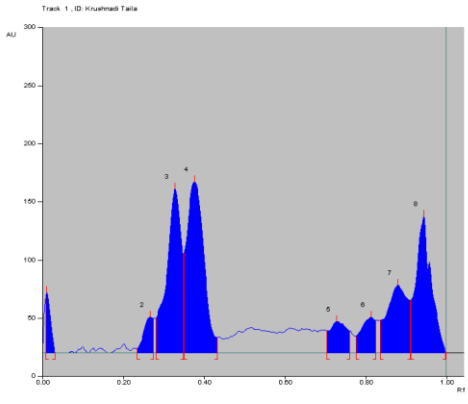

Fig 16: Densitogram of Methanol Extract of KT at 254 nm

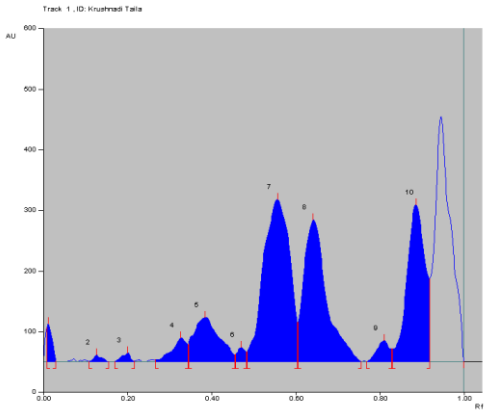

Fig 17: Densitogram of Methanol Extract of KT at 366 nm

The results shows that the active phytoconstituents are more sensitive for short UV radiation that is 254 nm when compared with 366 nm UV wavelength.

CONCLUSION

Preliminary Organoleptic features and results of powder microscopy were compared with the parameters mentioned in API and all the ingredients were proved to be authentic. In Phytochemical analysis RI, Acid Value, Saponification value, Iodine Value and Specific Gravity were assessed and all the values were in the normal range as per the standard values mentioned in API. Qualitative analysis revealed the presence of Tannins, Saponins, alkaloids, Glycosides, Carbohydrates and Flavonoids in the Taila. Though the base work requisites for the standardization of *KT* are covered in the current study, however additional analysis and investigations are required for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy.

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CONFLICTS OF INTEREST

No any personal or financial relationships that could be viewed as potential conflicts of interest in relation to the publication.

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