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Phytochemical screening and standardization of VachaTaila (A Herbal Ayurvedic oil based formulation) through HPTLC

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ABSTRACT
Ayurveda is a natural way for the medical science to manage herbs for various diseases which has been initially started in India a long time back. There are two Sanskrit words : ayur (life) and veda (science or knowledge), Ayurveda is the combination of them. So the significance of the word Ayurveda is "knowledge of life". According to Ayurveda, Vacha is a powerful medical herb to be used in the form of Nasya for various urdhavajatrugata disorders. The drug administration that belongs to Nasya is for the route of nasal cavity, which is also aimed in present medical studies for the screening of organoleptic analysis, physico-chemical analysis and phytochemical constituents through preliminary phytochemical tests of Taila. Vacha is referred to standardized this Vacha Taila as a High-Performance Thin Layer Chromatography (HPTLC) fingerprinting. Initial studies and screening with the methodology of Phytochemicals, which are chemical compounds produced by plants, to ensure that the screening extracts are revealing the presence of various bioactive compounds like flavonoids, alkaloid, tannin, steroids]triterpene ,carbohydrates and protein . This HPTLC fingerprint profile helps to obtain the authentic nasal Vacha Oil based formulation for further quality analysis.
Keywords: *Ayurveda, VachaTailal, oil based formulation, nasya, phytochemical analyses ,HPTLC fingerprinting, standardization.*

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INTRODUCTION

Industrialization, urban development and evolution in science and technologies change the ecosystem thus the Environment get more polluted. The way of life of man has changed. The man has failed to remember the nature and become reliant upon machine. The man becomes less vaccinated. Due to natural pollution, global warming, unnatural occasional variation, excessive utilization of A.C. and refrigerator, use of quick junk food and over the top nariprasang, and so forth an exceptionally normal and successive illness Pratishyaya happens in people. Pratishyaya is a vata-kaphajroga basically [1]. It is intently looking like as Rhinitis as depicted in present day clinical science.

Antiquated written works of Ayurveda incorporates very much depicted information on medication rehearsed for millennia and has its own library of valuable home-grown recipes. These medicinal plants are rich sources of beneficial constituents [2].

Siddha Taila (medicated/processed oil) is prepared by protracted boiling of the *Sneha Dravya*(base oil) with prescribed *DravaDravya*(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)[3].Thus, *Taila Paka Vidhi*(traditional method of Oil preparation) assures the enrichment of *Snehadravya* with the active principles of the ingredients.

VachaTaila is a Ayurvedic oil formulation used in several *Urdhvajatrugataroga* like in *Pratishyaya*[1,4]. Following are the ingredients used for the preparation of *VachaTaila*: (Table 2)

• *Sneha Dravya: Murcchita Tila Taila* (processed oil from seeds of *Sesamum indicum L.*)
• *Drava Dravya*: Cow milk
• *Kalka Dravya*: A paste obtained from the dried semi-powdered *vacha* rhizome.Quality control of Vacha Tailaremains an unexplored issue. Thus, in the present work an attempt has been made to use some newer approaches for the standardization of *Vacha Taila* with following objectives:
• To develop Standardized Operating Procedure (SOP) for the preparation of *Vacha Taila*.
• To evaluate the Organoleptic,physic - chemical analysis , phytochemical identification and safety profile of *Vacha Taila.*
• To carry out chemical characterization of Vacha 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 *VachaTaila*[7].

-*Vacha Taila*was prepared at GMP Certified- Parul Ayurved Pharmacy, Parul University, Limda ,Vadodara,Gujarat.
The SOP for the preparation of *VachaTaila* involved following steps:

- Preparation of *Murchita Tila Taila*[6,7]as per (Table 1):

In the preparation of *murchitaTila Taila*(*tilatailamurchha*), 1.8 litter of Til Taila was taken and 1/16 part of it , *Manjistha* was taken.Then*Musta, Haridra, Ushira, Lodhra, Ketaki,(Vata)Nyagrodha,KamalNaluka ,and Triphala (Haritaki, Bibhitaki, Amalaki)each drugs were taken as fourth part of Manjistha in (Yavakut) dried semi- powdered form .Water was taken as four part of Tilataila(7.2 litter approx.)* The Til Taila 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*.[7] (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 1.5liters was obtained by filtration . The final Murchita Til Taila was obtained.

- Preparation of *Kalka*: Each *Kalka Dravya* (375 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 *VachaTaila* :as per (Table 2),*MurchitaTilaTaila*(1.5 liters) was indirectly heated on a mild flame with , *VachaKalka Dravya*, (375 grams drug.) Mixture was stirred intermittently till it became slimy.

The heating was stopped and Cow Milk of amount 1.5liters was added. The mixture was kept standing overnight. Next day, the heating was continued till the mixture attained *Sneha SiddhiLakshana*[6,7].(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 *MurchitaTila Taila* (*Tila Taila Murchha*).

Sl. No.	Ingredients	Latin Name	Part Used	Quantity
1	*Manjishtha*	Rubia cordifolia Linn.	Stem	112.5 grams
2	*Ushira*	Vetiveriazizanoids	Root	28.125grams
3	*Haridra*	Curcuma Longa	Rhizome	28.125grams
4	(*Vata*)*Nyagrodha*	Ficus benghalensis L.	Root -Aerial	28.125grams
5	*Ketaki*	Pandanus Fectonussoland	Stem	28.125grams
6	*Kamala Naluka*	Nelumbo nucifera Gaertn.	Stalk	28.125grams
7	*Musta*	Cyperus rotundus L.	Rhizome	28.125grams
8	*Lodhra*	Symploeos racemose Roxb.	Stem Bark	28.125grams
9	*Haritaki*	Terminalia chebula Retz.	Fruit	28.125grams
10	*Bibhitaki*	Terminalia bellericaRoxb.	Fruit	28.125grams
11	*Amalaki*	Emblica officinalis (Gaertn.)	Fruit	28.125grams
12	*Krishna Tila Taila*	Sesamum indicum L.	Sesame oil	1.8 liters
13	*Jala*	Water	-	7.2 liters

Table 2: List of ingredients for the preparation of Vacha Taila

Sl. No.	Ingredients	Latin Name	Part Used	Quantity
1	*Vacha*	Acorous Calamus	Rizome	375gm
2	*Tila Taila (Murcchita)*	Sesamum indicum L.	Processed Sesame oil	1.5liters
3	*Go Dugdha*	Cow Milk	Cow Milk	1.5 liters

Phytochemical analysis[8,9].

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.

High Performance Thin Layer Chromatography[10,11].

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 [Vanillin – sulphuric acid reagent]

Dissolve 50mg Vanillin in 2 ml Methanol and 8 ml Sulphuric acid (98%). 4.0 µl 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 CAMAG Linomat5 TLC applicator . The plate was developed in Petrolium ether : Diethyl ether : Acetic acid (9 : 1 : 0.1 v/v/v). The developed plates were visualized in short UV 254, 366, and then derivatised with Vanillin-Sulphuric acid reagent and scanned under UV 254nm, 366 nm and 540 nm. Rf and densitometric scan were recorded.

RESULTS AND DISCUSSION

Organoleptic and Physico-chemical Analysis

Organoleptic and Physico-chemical characters of "*Vacha Taila"*are illustrated in (Table 3). The description provides as Dark yellow to brown coloured*Taila* (Oil) having characteristic odour. The obtained drug have Specific gravity as 0.953146 and Refractive index as 1.3220.The drug having Acid value as 6.03.

High Performance Thin Layer Chromatography[10,11].

HPTLC photo documentation of "*Vacha Taila*" (Fig -1) showed Five, Eight and Four spots under 254 nm, 366 nm and 540 nm after derivatization respectively. Spot with Rf value 0.08,0.23 and 0.94were commonly detected in any two detection methods. Spot with Rf value 0.11 and 0.18 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 1high peak and 2 peaks corresponding to 5 different compounds in the methanol extract, compounds with Rf value 0.11, 0.18, 0.23 were the high peaks (Fig- 1). At 366 nm there were Four high peak and Four low peak corresponding to 8 different compounds in the methanol extract, with Rf value 0.11,0.23,0.89,0.94 being the major peak detected (Fig- 2). At 540 nm there were Four peaks and Two high peaks, with Rf value 0.11 and 0.18 being the major peaks detected (Fig- 3).

Preliminary Phytochemical Tests

The phytochemical screening results showed the presence of Alkaloids, Tannins , Flavonoids , Steroids , Triterpenes, Proteinsand Carbohydrates in the extract of drugs of "Vacha Taila"(Table 4). Most of the identified phytochemical compounds have been reported to have various biological activities like Triterpenes[13,14,15,16]have anti-inflammatory, antiviral, antimicrobial, and antitumoral agents, as well as being immunomodulator compounds. The mechanism behind the virucidal activity was suggested to be direct inactivation of free viral particles. Triterpenes inhibites the expression of inflammatory factors secreted by Human Mast Cells (HMC-1) induced by Phorbol 12-Myristate 13- Acetate (PMA) and calcium carrier A23187. In the animal model of allergic rhinitis induced by Ovalbumin (OA), the scores of friction, histamine, IgE, inflammatory factors and inflammatory cells decreased after triterpene was administered orally or nasally.

Alkaloids[17]Several pharmacological properties are attributed to alkaloids as a result of their antibacterial, antiproliferative, and antioxidant effects. It also possess anti-inflammatory, antioxidant properties, have benefit in antipyretic,analgesic, antihypertensive etc. (histamines prompt thin walls, called membranes, to make more mucus. We can get arunny or stuffy nose. And we'll sneeze. The mucus can also bother your throat and make you cough.)

Alkaloids reduced the production of IgE, one of the immune molecule responsible for mast celldegranulation and also, slight, reduced the number of mast cells in conjunctive tissue next to the nasal cavity of animals with rhinitis. Alkaloids treatment decreased mucus production in nasal proximal squamous epithelium, resulting in decreasing of hyperplasia and hypertrophy of goblet cell.

Steroids[18]Plant steroid have anti-inflammatory property.It is used in the management of inflammatory disorders such as asthma, rheumatoid arthritis, rhinitis, conjunctivitis and multiple sclerosis.

Tannins[19] are virucidal compounds in the sense that they attack the membrane wall of the virus. the mode of action is one of destroying viruses on contact. While the virucidal properties of tannins in the form of tannic acid .

Tannic acid displays a wide range of pharmacological activities, such as anti-inflammatory, neuroprotective, antitumor, cardioprotective, and anti-pathogenic effects.

Flavonoids [20]have been found to have several biological effects ,that is antioxidant ,anti-inflammatory, anti-carcinogenic, anti-obesity ,anti-diabetic and immunemodulating and also to possess anti-allergic properties.

Flavonoids can inhibit regulatory enzymes or transcription factors important for controlling mediators involved in inflammation. Flavonoids are proposed to reduce the incidence of upper respiratory track infections because they have a range of physiologic effects in humans, including antiviral, anti-inflammatory, cytotoxic, antimicrobial, and antioxidant.

Studies report flavonoids have both an antiproliferative and antireplicative effect on two common viral sources of upper respiratory track infections and reduce inflammation by decreasing NF-κB. These mechanisms, and others, may have the potential to decrease upper respiratory track infections incidence, which makes flavonoids a current field of interest in human immunity.

Flavonoids are also known as potent antioxidants with the potential to attenuate tissue damage or fibrosis. Consequently, numerous studies ***in vitro*** and in animal models have found that flavonoids have the potential to inhibit the onset and development of inflammatory diseases.

Carbohydrates[21,22]During cell-pathogen interactions (i.e. ,invasion or infection) carbohydrates function as receptors for various pathogens.

Allergic rhinitis was significantly correlated with high-fat and low-carbohydrate diets.

Proteins[23]**.**Inflammation was indicated by α1- and α2-globulins, a weak immune response, and IgM and IgG. High oxidative stress and low antioxidative ability of blood serum were observed. The plant-based protein product (FP) helped preserve testosterone level and prevent an increase in catabolic reactions. Moreover, it had a positive effect on both red blood cell hematopoiesis (a smaller increase in the average volume of erythrocytes, the same average concentration and content of hemoglobin, an increased relative red cell distribution width (RDW) and white blood cell hematopoiesis (a beneficial effect for the immune system: lymphocytes, the relative content of neutrophils, monocytes, basophils and eosinophils). The stimulation of humoral immunity was evidenced by beta- and gamma-globulins, an active immune response, the level of IgM and IgG, antioxidant protection, reduction of peroxides and an increase in antioxidant activity of blood serum.

Table 3: Organoleptic and Physico-Chemical Analysis of Vacha Taila

Sr.No	Parameters	Results
1	Description	Dark yellow to Brown coloured oil
2	Odour	Characteristic
3	Specific gravity	0.953146
4	Saponification value	235
5	Refractive index	1.3220
6	Acid value	6.03
7	LOD	0.95
8	Ash Value	0.002

Table 4 : Phytochemical constituents of *VachaTaila*

Sr. No.	Parameters	Results
1	Alkaloid	+
2	Starch	-
3	Tannins	+
4	Saponins	-
5	Flavonoids	+
6	Carbohydrates	+
7	Proteins	++
8	Steroids	++
9	Triterpenes	++
10	Anthraquinine Glycosides	_
Key word: "+, ++, +++" indicates Present in increasing intensity and " - " indicates Absent.		

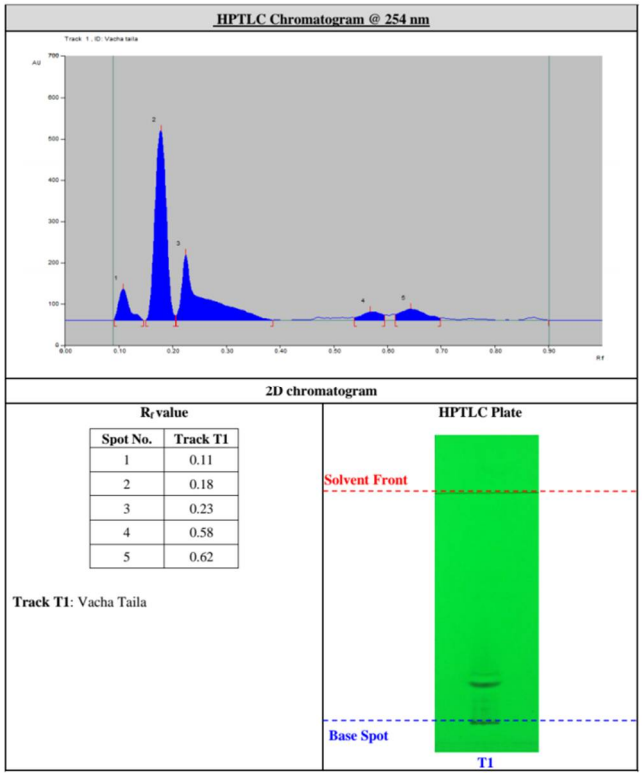


Fig.1. HPTLC plate showing banding pattern and Rf Values at 254 nm

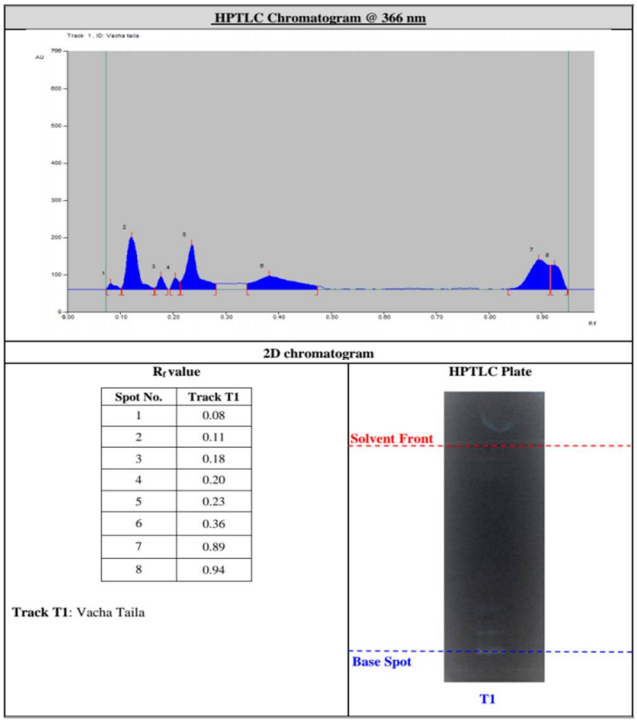
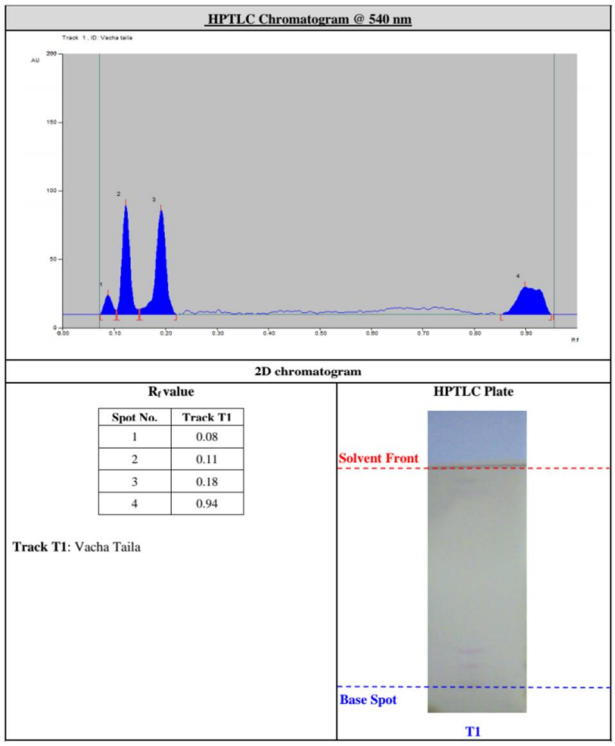


Fig. 2. HPTLC plate showing banding pattern and Rf Values at 366 nm



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

In the present work, *VachaTaila* is processed with cow milk, which is taken equal to proportion of *taila*. The purpose behind using cow-milk is to reduce *teekshnata* of *taila*. So that it should not irritate the nasal mucosa and not cause further irritation to patient. When clinically used on patient, cow-milk processed *VachaTaila* has given desired therapeutic effect without much irritation of nasal mucosa. It is an *Anubhut* Yoga as in classics we do not get the reference of addition of cow-milk in Vacha *Taila* . With the patient point of view by using *Yukti* and *Tarkapramantaila* has been processed with cow-milk.

CONCLUSION

Preliminary phytochemical tests of the extract of *VachaTaila* showed the presence of alkaloids, tannins, flavonoids, steroids, triterpenes ,carbohydrate and protein. which are reportedly bioactive in nature and may add up to the therapeutic effect of this nasal oil. HPTLC fingerprint profile of the Vacha Taila formulation may be used for authentication and quality control. So it can be concluded that these parameters can be used for the evaluation of *VachaTaila.* The present study can serve as the reference for the future works on *VachaTaila.*

CONFLICT OF INTEREST: None Declared.

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