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Phytochemical Screening and standardization of Vacha Nasal Drops through HPTLC in the management of Rhinitis

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ABSTRACT
Rhinitis is an inflammation and irritation of mucous membrane inside the nose. This inflammation is caused by viruses, bacteria, irritants or allergens and the common symptoms are stuffy nose, running nose, sneezing and post nasal drip .It is very common medical condition. Every man would have suffered from this disease at least once in his life. In Ayurveda Rhinitis is compared with Pratishyaya. According to Vagbhata Nasa being the gateway of shira any drug administered through this route reaches shrungatakamarma. This is a siramarma and formed by the siras of Nasa, Netra, Kantha and Shrotra etc. The elimination of dosha is quick and efficient by this route. In this study Vacha Nasal Drops used as Pratimasha Nasya.Vacha is a powerful medical herb to be used in the form of Nasya for various urdhavajatrugata disorders. Nasya belongs to administration of drugs by 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 Nasal Drops. Vacha is referred to standardized this Nasal Drops 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, carbohydrates, steroids and triterpenes. This HPTLC fingerprint profile helps to obtain the authentic nasal drops-based formulation for further quality analysis.
Keywords: *Ayurveda, Vacha Nasal Drops, Nasya, phytochemical analyses ,HPTLC fingerprinting, standardization*

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INTRODUCTION

Antiquated writing of Ayurveda incorporates all-around depicted information on medication rehearsed for millennia and has its own library of valuable homegrown equations. These therapeutic plants are rich wellsprings of helpful constituents.

Because of the advancement of science, industrialization and urbanization changed the environment, the climate gets dirtied. The way of life of man has changed. Man has failed to remember nature and become reliant upon machines. The man turns out to be less inoculated. Because of natural contamination, a dangerous atmospheric deviation, unnatural occasional variety, unnecessary utilization of A.C. also, coolers, utilization of quick Chinese food and unnecessary *Nari parasang*, and so forth an extremely normal and continuous sickness *Pratishyaya* happens in people. *Pratishyaya* is a *Vata-kaphajroga* for the most part[1,2].It intently looks like Rhinitis as portrayed in present-day clinical science. Rhinitis is an inflammation and irritation of mucous membrane inside the nose. This inflammation is caused by viruses, bacteria, irritants or allergens and the common symptoms are stuffy nose, running nose, sneezing and post nasal drip [3].

Vacha Nasal Drop is a Ayurvedic formulation used in several *urdhvajatrugataroga* like in *pratishyaya* [4]. Following are the ingredients used for the preparation of Vacha Nasal Drops.(Table 1)

• *Churnadravya: Vacha churna*
• *Drava Dravya*: Distilled water
Quality control of Vacha Nasal Drops remains an unexplored issue. Thus, in the present work an attempt has been made to use some newer approaches for the standardization of Vacha Nasal Drops with following objectives:
• To develop Standardized Operating Procedure (SOP) for the preparation of Vacha Nasal Drops.
• To evaluate the Organoleptic, physico- chemical analysis , phytochemical identification and safety profile of Vacha Nasal Drops.
• To carry out chemical characterization of Vacha Nasal Drops on the basis of active principles of the ingredients, using validated HPTLC method.

MATERIAL AND METHODS

Plant material

The ingredients 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 Vacha Nasal Drops[5,6].

-Vacha Nasal Drops was prepared at GMP Certified- Parul Ayurved Pharmacy, Parul University, Limda ,Vadodara, Gujarat.
The SOP for the preparation of Vacha Nasal Drops involved following steps:
Preparation of *Vacha* Nasal Drops[7,8]as per (Table 1): In step one *vachachurna* 150 gm was taken and socked in 1.5 litter distilled water for whole night (in 1:10 ratio).
●In Step two put the solution, taken from step one, after 24 hours in distillate machine.
●In Step three collect 700 ml of distillate from step two maintaining 70degree temperature of distillate machine.
●Outcome of step three is distillate having pH value 4, which is bellow than the preferred pH level (6to 7), which is not suitable for nasal mucosa.
The solution for this is to dilute distillate with distilled water in 2:3 ratio and obtained dilute distillate having pH 6.8.
●By this process obtained 1166ml of *Vacha* Nasal drops.

Table 1: List of ingredients for the preparation of *Vacha Nasal Drops*

S. No.	Ingredients	Latin Name	Part Used	Quantity
1	*Vacha*	*Acorous Calamus*	Rhizome	150grams
2	Distilled water			1500ml

PHYTOCHEMICAL ANALYSIS [9,10].

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 [11,12]

Preparation of Test Solution

10 g of sample was weighed accurately in a evaporating dish and evaporated till dryness. To it 5ml menthol was added and concentrated till 1 ml remains. Then after, filtered with help of 0.22micron. The filtrate 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 %). 15.0 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 Linomat 5- TLC applicator. The plate was developed in Toluene: Ethyl acetate : Acetic acid (7 : 3 : 0.1 v/v/v). The developed plates were visualized in short UV 254, 366, and 540nm then derivatised with Anisaldehyde 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 Nasal Drops" are illustrated in (Table 3). The description provides as transparent distillate having characteristic aromatic odor. The obtained drug have Specific gravity as 0.99854 and Refractive index as 1.3190 .The drug having Viscosity by Ostwald as 37.1 cP,

Table 3: Organoleptic and Physico-Chemical Analysis of VachaNasal Drops

Sr.No	Parameters	Results
1	Description	Transparent
2	Odour	Characteristic aromatic smell
3	Specific gravity	0.998549
4	Viscosity by Ostwald	37.1 cP
5	Refractive index	1.3190
6	pH value	6.6
7	Ash value	0.002

Table 4 : Phytochemical constituents of Vacha Nasal Drop

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

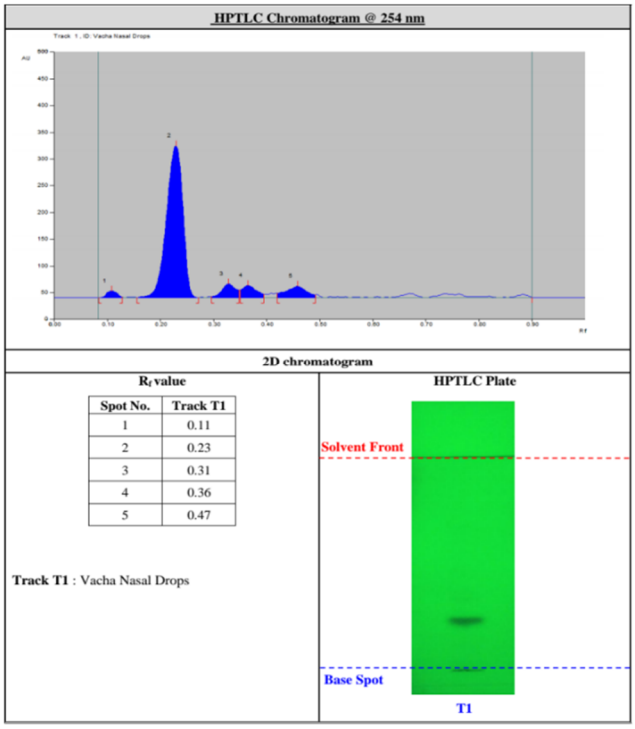

Fig.1. HPTLC plate showing banding pattern and Rf Values at 254 nm

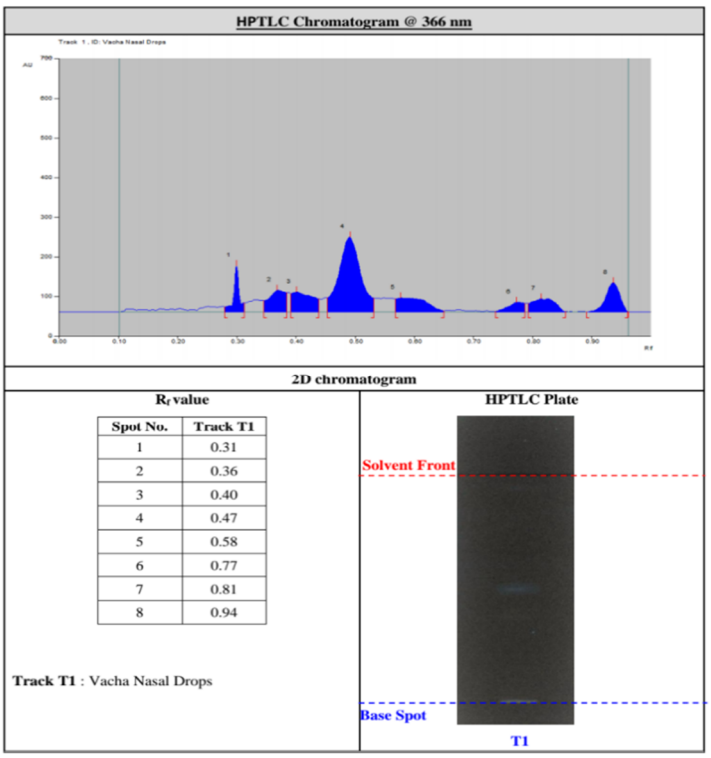

Fig. 2. HPTLC plate showing banding pattern and Rf Values at 366 nm

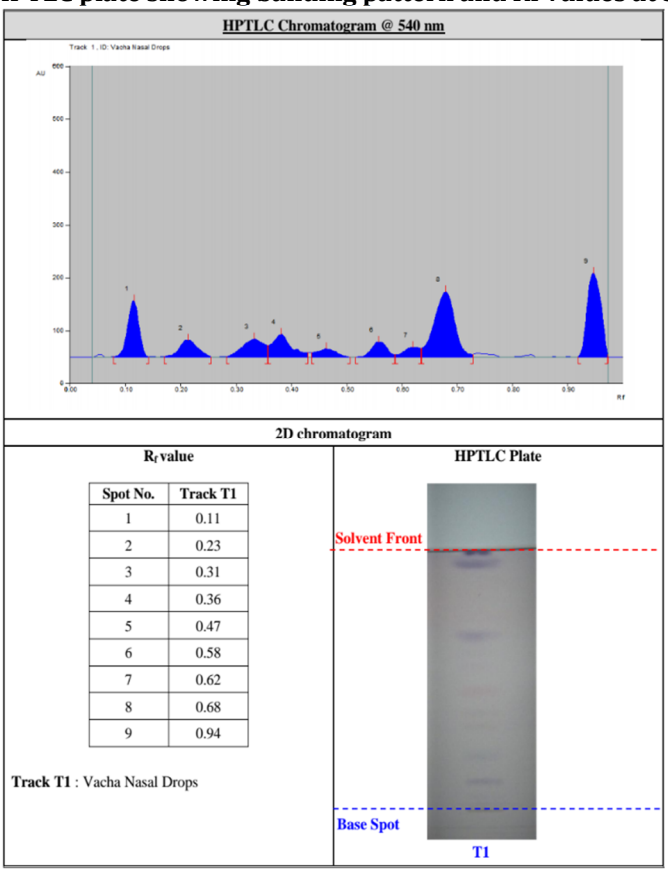

Fig. 3. HPTLC plate showing banding pattern and Rf Values at 540 nm

Preliminary Phytochemical Tests

The phytochemical screening results showed the presence of Alkaloids, Flavonoids ,Triterpenes, Steroids and Carbohydrates, in the extract of drugs of "Vacha Nasal Drops". Most of the identified phytochemical compounds have been reported to have various biological activities like -

Triterpenes (TRI)[13,14,15,16,17] has significant pharmacological activities, such as antiviral, antimicrobial, anti-inflammatory, immunosuppressive and anti-tumor activities.
Triterpenes used to treat allergic diseases because of its characteristics of immunosuppression. Triterpenes ,as an active immunoregulatory factor, has great potential in the treatment of mast cell-mediated allergic diseases.
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 **frictions**, histamine, IgE, inflammatory factors and inflammatory cells decreased after triterpene was administered orally or nasally.
Carbohydrates [7,17]-During cell-pathogen interactions (i.e., invasion or infection) carbohydrates function as receptors for various pathogens.
Alkaloids[18]-In plants alkaloids protect plants from predators and regulate their growth .Therapeutically, alkaloids are particularly well known as anasthetics, cardioprotective , and anti-inflamatory agents. Alkaloids are use as antipyretic, analgesic, antihypertensive etc.
(Histamines prompt thin walls, called membranes, to make more mucus. We can get a runny 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 **molecules** responsible for mast cell degranulation 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: 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.
Flavonoid [15] 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 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
High Performance Thin Layer Chromatography [12,13]
HPTLC photo documentation of "*Vacha* Nasal Drops(Fig -1) showed Five, Eight, and Nine spots under 254 nm, 366 nm and 540 nm after derivatization respectively. Spot with Rf value 0.11, 0.23, 0.94 and 0.58 ,were commonly detected in any two detection methods. Spot with Rf value 0.31 , 0.36 and 0.47were 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 1 high peak and 4 small peaks corresponding to compounds in the methanol and sulphuric acid extract, compounds with Rf value 0.23 was the high peak (Fig- 1). At 366 nm there were three high peaks and five low peak corresponding to 8 different compound in the methanol and sulphuric acid extract, with Rf value 0.31,0.47 ,0.94, being the major peak detected and Rf value 0.36,0.40,0.58.0.77,0.81 were the small peaks (Fig- 2). At 540 nm there were nine peaks and three high peaks, with Rf value 0.11,0.68, 0.94 being the major peaks detected and 0.23,0.31, 0.36, 0.47, 0.58,0.62 were small peaks (Fig- 3).

CONCLUSION

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

Conflict of interest None Declared.

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