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PRELIMINARY PHARMACOGNOSTICAL AND PHYSICOCHEMICAL ANALYSIS OF PATHYADI VARTI (PVN 1): A HERBOMINERAL FORMULATION

Gupta Varun B[1]*, Harisha CR[2], Manjusha R[3], Ravishankar B[4]

[1]Pharmacology Lab, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

[2]Head, Pharmacognosy Laboratory, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

[3]Associate Professor, Dept of Shalakya, Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

[4]Director, Research and Development, Allied Science of Medicine, SDM College of Ayurveda, Udupi, Karnataka, India

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***Corresponding author**

Dr. Varun B. Gupta, Ph D (sch), Pharmacology. Institute for Postgraduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat – 361 008 India Email: varunn999@yahoo.co.in

ABSTRACT

Present study evolves a systematic approach and to develop well designed methodologies for the standardization of Pathyadi varti (PVN 1), an Ayurvedic herbomineral formulation. The varti is consists of Pathya, Tuttha, Yashtimadhu and Maricha. The finished product was subjected to organoleptic study, microscopic characterization, physico-chemical screening, phyto-chemical analysis and HPTLC. The pharmacognostical evaluation shows fragments of mesocarp cells, sclereids, starch, stone cells and tannin content from Pathya (Haritaki); lignified fibers, fibers with crystals, prismatic crystals, pitted vessels, and larger starch grains of Yashtimadhu; simple fibers, beaker shaped stone cells and oil globules from Maricha. The Phytochemical analysis shows the presence of alkaloids, tannins, flavonoids, saponins and anthraquinon glycosides. Spots obtained in HPTLC were found resembling spots of glycyrrhizin at R_f 0.27 and piperine at R_f 0.40 as reported in previous studies.

KEYWORDS: Analytical study, Ayurveda, Pathyadi varti, Pharmacognosy, Physicochemical, HPTLC.

INTRODUCTION

Ayurveda is well known traditional medicine system practiced in India for several centuries.[1] Herbal medicines are being used by about 80% of the world population mainly in the developing countries, for primary health care.[2] The plant species mentioned in the ancient texts of Ayurveda and other Indian Medicine Systems may be explored with the modern scientific approaches for better leads in the healthcare. Standardization of herbal formulation is essential in order to assess the quality of drugs.[3,4] Each ingredient of polyherbal formulation has to be established. Usually, the microscopic characters of each ingredient in such formulations are very difficult to identify and also some times these are overlapping with the character of other ingredients. The WHO has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicines and to study their potential usefulness including evaluation, safety and efficacy.[5] The manuscript presents development of methods for the evaluation of Pathyadi varti, an Ayurvedic herbomineral formulation, used for various eye diseases viz; cataract, trachoma etc. It consists of fine powder of Pathya (Haritaki), Tuttha, Yashtimadhu and Maricha.[6] The reports on the standardization of Pathyadi varti, are based on organoleptic, microscopic, physico-chemical, phyto-chemical parameters and HPTLC study.

MATERIALS AND METHODS

Collection of the raw drug

Raw herbal samples viz; fruits of Haritaki (*Terminalia chebula* Retz), roots and stolons of Yashtimadhu (*Glycyrrhyza glabra* Linn), fruits of Maricha (*Piper nigrum* Linn.) were purchased from the local market of Kerala, India. Raw Tuttha ($CuSO_4$) was procured from the Pharmacy, I.P.G.T. and R.A., Jamnagar, India.

Authentication of raw drugs

Raw drug samples deposited to the **'Raw Drug Museum'** under Pharmacognosy Lab, IPGT and RA, Gujarat Ayurved University, Jamnagar. Their Herbarium voucher number noted as 6033, 6034 and 6035 towards Haritaki, Yashtimadhu and Maricha respectively. The identities and authentication of raw drugs, confirmed by correlating their morphological and microscopical characters with those given in literature.[7-10]

Preparation of test drug

The obtained fruits and root-stolons were shade dried and made in to fine powder separately with the help of mechanical grinder, sieved through 85# and stored in airtight containers. Raw tuttha was dissolved in sufficient quantity of distilled water. This solution filtered, dried until obtained the clear crystals and converted into fine powder. All the fine powders where taken in porcelain mortar, mixed well and added in wet grinder (triple granite stone) and sufficient quantity of cold water was added. Grinding was done till semi solid consistency was obtained. This process was repeated for three days. Vartis were prepared of homogenous sizes as per described in Ayurvedic classics.[6]

Organoleptic Evaluation

Colour, odour, taste, touch and appearance of the finished product (varti) observed and documented..[6]

Microscopic Evaluation

Sample drug powdered, dissolved in a small amount of distilled water for a brief duration and then mounted in glycerine. Microscopical examination was carried out with and without staining.[11] By powder microscopy, to observe the characters, determined the histo-chemical nature of the cell wall. Microphotographs were taken by using Carl Zeiss binocular microscope attached with camera.[12]

Physico-chemical Constants

Foreign matter, hardness, moisture content, ash values viz., total ash, acid insoluble ash and extractive values viz., alcohol soluble extractive value, water soluble extractive values as well as pH value were determined by adopting standard methods.[13]

Phyto-chemical Analysis

Preliminary tests were carried out on methanolic extract for the presence or absence of phytoconstituents like alkaloids, tannins and phenolic compounds, flavonoids, saponins and anthraquinone glycosides.[14,15]

High Performance Thin Layer Chromatography

HPTLC was performed as per the guidelines provided by API.[16] Methanolic extract of drug sample was used for spotting. HPTLC was performed using Toluene + Ethyl acetate + acetic acid (7:2:1) solvent system and observed under visible light after derivatization with vanillin sulfuric acid followed by heating the plate at 110°C. The colour and R_f values of the resolved spots were noted. (Table 5)

RESULTS AND DISCUSSION

Organoleptic Characters

Finished product characterized as dark yellowish green in colour (rupa), pungent in taste (rasa), characteristic of piper-aromatic in odour (gandha), smooth touched (sparsha) and solid varti (yavakaar) in appearance (Table 2).

Microscopical Characters

Diagnostic characters of microscopic analysis of test drug shows the presence of mesocarp cells, elongated pitted sclereids, small sized starch grains and spherical pitted stone cells indicated the presence of *Terminalia chebula*. Fragments of mesocarp cells, beaker shaped stone cells, stone cells intercepted with parenchyma cells and fraction of volatile oils indicated the presence of *Piper nigrum*; fragments of epidermal cells, pitted vessels, lignified fibers, fibers with crystals, prismatic crystals, and larger sized starch grains with concentric hilum indicate the presence of *Glycyrrhyza glabra* Linn. (Photo Plate 1)

Table 1: Ingredients of *Pathyadi Varti*

SN	Name of Ingredients	Parts used	Ratio
1	Haritaki	Fruits	1 part
2	Tuttha (Nirmalikrita)	Crystals	1 part
3	Yashtimadhu	Roots and stolons	1 part
4	Maricha	Fruits	16 part

Table 2: Organoleptic Characters

SN	Parameters	Results
1	Colour	Dark yellowish green
2	Odour	Characteristic-aromatic
3	Taste	Pungent
4	Touch	Smooth
5	Appearance	Solid

Table 3: Showing Physicochemical Constants

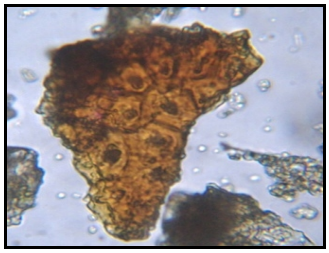
SN	Parameters	Results
1	Foreign matter	Nil
2	Hardness	5.2-5.5 kg/cm^2
3	Loss on Drying	8.69%
5	Total Ash content	7.88% w/w
6	Acid insoluble ash	0.97% w/w
7	Alcohol soluble extractive value	14.60% w/w
8	Water soluble extractive value	11.20% w/w
9	pH value	4.0

Table 4: Showing results of Phytochemical Analysis

SN	Components	Results
1	Tannin and Phenolic compounds	Present
2	Alkaloids	Present
3	Saponin Glycosides	Present
4	Flavonoid	Present
5	Anthraquinone glycosides	Present

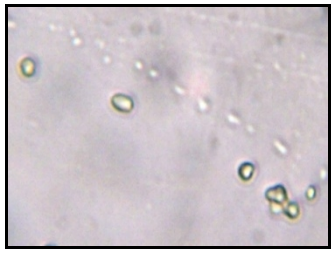
Table 5: Results of HPTLC study of *Pathyadi varti*

No of spots	Rf values of methanolic extract of *Pathyadi varti*	
	254 nm	366 nm
1	0.05	0.05
2	0.13	0.13
3	0.26	0.30
4	0.40	0.48
5	0.57	0.51
6	0.65	0.54
7	0.73	0.57
8	0.79	0.65
9	0.91	0.74
10	--	0.96

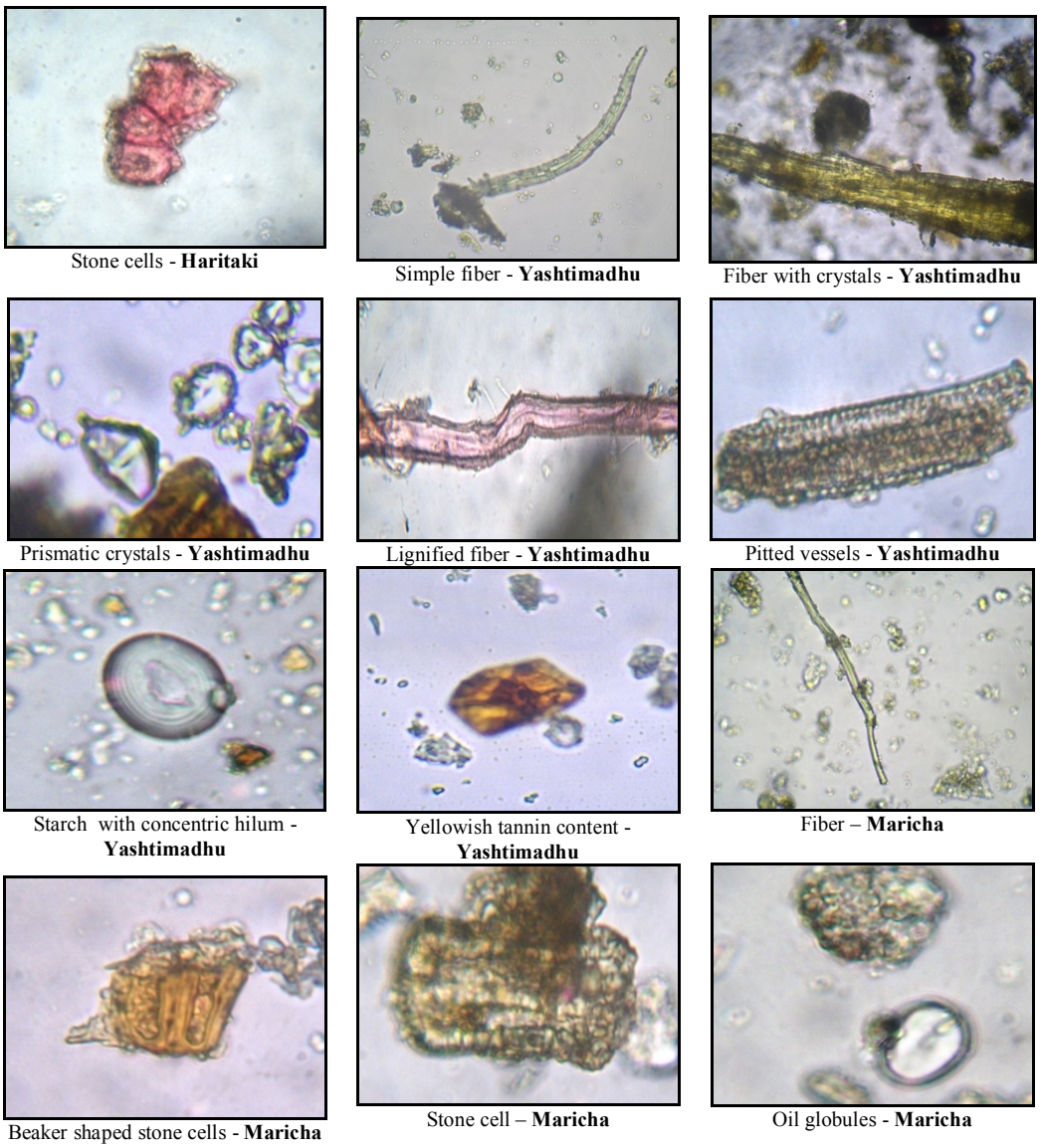


Fragment of Mesocarp cells - **Haritaki**

Sclereid - **Haritaki**



Starch grains – **Haritaki**



Stone cells - **Haritaki**

Simple fiber - **Yashtimadhu**

Fiber with crystals - **Yashtimadhu**

Prismatic crystals - **Yashtimadhu**

Lignified fiber - **Yashtimadhu**

Pitted vessels - **Yashtimadhu**

Starch with concentric hilum - **Yashtimadhu**

Yellowish tannin content - **Yashtimadhu**

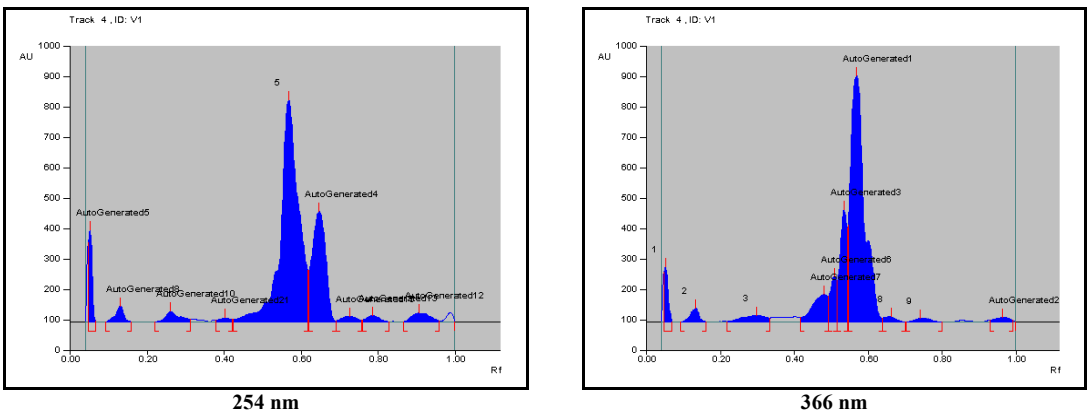
Fiber – **Maricha**

Beaker shaped stone cells - **Maricha**

Stone cell – **Maricha**

Oil globules - **Maricha**

Photo Plate: 1 - Powder Microscopy of *Pathyadi varti*



254 nm **366 nm**

Photo Plate 2: Densitograms of Methanolic extract of *Pathyadi varti*

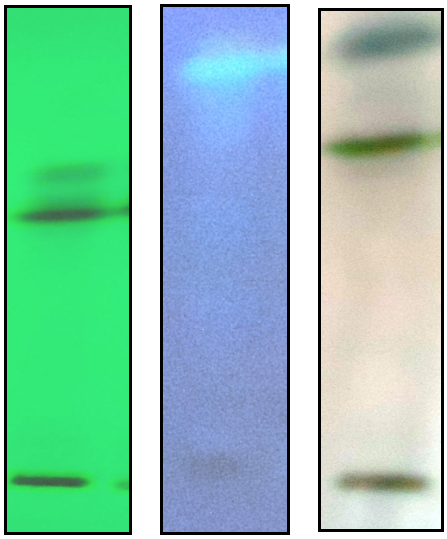


Photo Plate 3: Visualization of Methanolic extract of *Pathyadi varti*

Physico-chemical Parameters

Physio-chemical parameters of Pathyadi varti are tabulated in Table 3. The hardness of the varti is within the acceptable range of 5.2-5.5 kg/cm^2. Loss on drying at 110°C is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The results of loss on drying at 110°C of prepared vartis showed the lower limits than the prescribed in API. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparation of the formulation. The results of ash value revealed that the preparation have lower value than mentioned in API. Water soluble and alcohol soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. The extractive values of preparation were observed more in alcohol then water. pH showed the acidic nature of the formulation, though compatible for application to the eye.

Phyto-chemical analysis

Preliminary qualitative analysis showed the presence of tannin and phenolic compounds, alkaloids, saponin glycosides, flavonoids and anthraquinone glycosides indicating the active compounds were not disturbed during the preparation. (Table 4)

HPTLC

The methanolic extract of the formulation was subjected to HPTLC. Results revealed from table 5, indicating the presence of all major constituents as per the ingrediants. During the observation of HPTLC plate of test formulation under Short UV light and long UV light 9 and 10 spots obtained respectively. Short UV light and long UV light spectrum showing some of the identical spots at R_f 0.05, 0.13, 0.57 and 0.65. The spot at 0.26 may be recognised as glycyrrhizin[17] in short UV and 0.40 as piperine[18] in short UV light spectrum as reported in various studies. HPTLC of the test drug after derivatization with vanillin sulfuric acid reagent showed two major spots. (Photo Plate 2 and 3)

CONCLUSION

After analysis of Pathyadi varti by different parameters like organoleptic, microscopical, physicochemical, phytochemical and HPTLC densitograms show good co-relation between them and are similar as per the previous reported works. The study of microscopic characters of present formulation shows the presence of diagnostic identifying characters of ingredients which are used. Purity and potency of the materials and formulations following the procedures given could be performed in QC/QA laboratory of Pharmaceutics. The present study can serve as the reference for the future works on Pathyadi varti.

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