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ORIGINAL RESEARCH ARTICLE

Pharmaceutico – Evaluation of *Pathyadi Varti Anjana* Containing Moringa Oleifera Seeds: An Analytical Perspective

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ABSTRACT

Background: Blur vision, particularly due to cataracts, remains a significant global public health concern, especially in rural and underserved populations. Ayurvedic formulations such as *Pathyadi Varti* (PV), which comprises four potent herbo-mineral ingredients with psycho-physical *Kaphahara* properties, have shown promise in addressing age-related ocular degeneration. This study evaluates the physicochemical properties, organoleptic characteristics, and phytochemical composition of PV.

Material and Methods: PV was prepared following classical Ayurvedic guidelines. Analytical evaluations included organoleptic and physicochemical assessments, qualitative phytochemical screening, and high-performance thin-layer chromatography for the detection.

Results: Results highlighted PV's quality, stability, and therapeutic potential. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, proteins/amino acids, and saponins.

Conclusion: These findings underscore the potential of PV as a complementary therapeutic agent in cataract management. Its antioxidant properties, combined with the presence of key phytochemicals, suggest a role in mitigating oxidative stress, a key contributor to cataract formation. Integrating such formulations into modern therapeutic strategies may address unmet needs in managing age-related visual impairment.

1. INTRODUCTION

Vision is the most crucial human sense, utilizing over 30% of the brain's processing capacity. Consequently, the fear of losing eyesight ranks among the top health-related anxieties.[1] Globally, cataracts are the leading cause of treatable blindness. In India, they account for blindness in approximately 0.73% of the population.[2] Research suggests that around 14.25% of older adults in India are affected by cataracts, with higher rates found among women and the elderly.

Studies show notable differences in cataract prevalence between rural and urban populations in India. One study reported cataracts in 41.3% of rural and 38.6% of urban individuals. Monotype cataracts appeared in 32% of the rural group and 25% of the urban group, whereas mixed cataracts were found in 12.68% and 18.6%, respectively. Age is a key risk factor across both groups. Lower socioeconomic status is linked to higher cataract prevalence, especially in urban areas. In rural settings, higher HbA1c levels – an indicator of diabetes – are also associated with increased cataract risk.[3] Cataracts remain a major public health challenge globally and in India. While surgical treatments are expanding, unmet needs persist, particularly among certain groups and in rural areas. Bridging these gaps is essential for reducing cataract-related vision loss.

From an Ayurvedic perspective, senile cataracts align with "*Jara Janya Vyadhi*" (age-related conditions), reflecting ancient insights into age-associated degeneration. Ayurveda proposes various treatments known for promoting ocular health through their "Chakshushya" (eye-beneficial) properties. One such Ayurvedic formulation is *Pathyadi Varti* (PV), comprising four powerful herbo-mineral PV have four

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herbo-mineral drugs [*Terminalia chebula* Retz., *Glycyrrhiza glabra* Linn., *Moringa oleifera* (seeds) Lam. Copper sulfate ($CuSO4.5H_2O$) is known for its holistic benefits, including support for age-related eye disorders and adaptogenic effects. Clinical findings suggest that this formulation can help slow the progression of senile immature cataracts by leveraging its broad pharmacological benefits.

However, due to the complex and variable nature of herbal-mineral compositions, ensuring consistent quality, safety, and efficacy is a significant challenge. Advanced analytical methods are, therefore, vital for quality control and validation, supporting its potential integration into modern health care and improving global acceptance.

2. MATERIALS AND METHODS

2.1. Collection and Authentication of Raw Drugs

Ingredients of PV were procured from the Pharmacy of All India Institute of Ayurveda, New Delhi. Identification and authentication of individual ingredients were done in the Pharmacognosy Laboratory of RRDR, AIIA, New Delhi, with authentication numbers

RRDR/AIIA/phg./343, RRDR/AIIA/phg./346, RRDR/AIIA/phg./354. RRDR/AIIA/phg./355.

2.2. Macroscopic Study

The plant parts of interest were spread on the table and examined for their appearance, color, texture, and dimension.

2.3. Powder Microscopy

All the ingredients of PV were ground into fine powders using a mixer and grinder and sieved through a #80 mesh. A pinch of fine powder was added to tap water with 2% chloral hydrate and heated at 80°C for 12 h. Three slides were prepared: safranin-stained, iodine-stained for starch grains, and phloroglucinol-hydrochloride acid-stained for lignified tissues. Glycerine was added, covers lipped, and observed under a microscope at various magnifications, with photographs taken.

2.4. Method of Preparation of *Pathyadi Varti Anjana*

The ingredients mentioned in Table 1 were individually washed to remove impurities and then dried naturally. After cleaning the *Tuttha*, it was dissolved with the help of a stirrer in RO water, and then the *Tuttha*-containing water was filtered through filter paper. The filtered water was boiled again to obtain *Tuttha* crystals.[4] *Tuttha* crystals were grinding in a mortar with the help of a pestle, and then by adding lemon juice 3 times, purified *Tuttha* was obtained, and then it was used to make *Varti*.[5] After complete drying, all the ingredients were pulverized into a fine powder. The powder was placed in a mortar and triturated seven times with cold water to prepare the *Varti.* This semisolid mass was then manually rolled *Varti*, each weighing 120±4 mg. The *Varti* were dried in the shade to preserve their potency and medicinal properties. Finally, the dried *Varti* were stored in an airtight container to ensure their stability and efficacy.[6]

2.5. Physico-chemical Analysis

Quality control parameters such as organoleptic tests (color, odor, touch, consistency, etc.) and physicochemical parameters such as pH (using a digital pH meter with a 10% aqueous solution),[7] loss on drying at 105°C (moisture content),[8] friability, hardness, uniformity of weight, disintegration time, total ash value,[9] acid-insoluble ash,[10] alcohol-soluble extractives,[11] water-soluble extractives,[12] foreign matters were performed as per standard guidelines.

2.6. Phytochemicals Analysis

Aqueous and methanolic extracts were prepared for all samples following the standard method for the preliminary phytochemical analysis.[13] Both of these extracts of three samples were subjected to various qualitative tests to determine the presence of alkaloids, proteins, flavonoids, tannins, and saponins [Table 4].[14]

2.7. Microscopic Evaluation of PV

The microscopic evaluation of PV involves examining a powdered sample under a microscope to identify the diagnostic characteristics of its ingredients for quality control and authentication. A small amount of powder is mounted on a slide with glycerin and stained with reagents such as safranin, iodine, or phloroglucinol with HCl to highlight specific features. Prepared slides were observed under magnification of low (×10) and high (×40). Images of significant diagnostic features were captured using a microscope camera to record the observations.

2.8. High-Performance Thin Layer Chromatography (HPTLC) Analysis

2.8.1. Chemicals and reagents

High-performance liquid chromatography-grade chemicals, including HCl, methanol, ethanol, toluene, chloroform, and glacial acetic acid Merck grade were used.

2.8.2. Sample preparation

1 g sample of the PV was taken, and 10 mL methanol was added to it. The mixture was shaken for 6 h and then kept overnight. The next day, it was filtered through Whatman filter paper no. 41 into a 10 mL volumetric flask. This solution was used for HPTLC analysis.

2.8.3. Instrumentation and chromatographic condition

The test solutions were applied as bands using a CAMAG Linomat 5 with 100 µL Hamilton syringe, a semi-automatic sample applicator on HPTLC silica gel 60 F_{254} (0.2 mm thickness, E. Merck) under a nitrogen stream. The parameters were: Band length: 8 mm, dosage speed: 150 nL/s, and pre-dosage volume: 0.2 µL. The plates underwent linear ascending development up to 70 mm from the base in an optimized solvent system within a CAMAG TLC Twin Through Chamber equipped with a stainless-steel lid, previously saturated with the appropriate mobile phase, Toluene:Ethyl acetate:Glacial acetic acid (7:2:1, v/v/v) employing saturation pad for 20 min at room temperature. The volume of the solvent system was evenly distributed between the front and rear sections of the chamber. After proper development, the plate was dried at room temperature for 5 min using an air dryer and visualized in white light at λ 254 nm (short UV) and λ 366 nm (long UV) in TLC Visualizer 2. Deuterium and Tungsten lamps were used as radiation sources for the respective wavelengths. Finally, the plate was sprayed with Anisaldehyde Sulfuric Reagent (ASR) and heated in a hot air oven at 100°C for 3 min. The derivatized plate was photo-documented under white light and scanned using CAMAG TLC scanner 4 with Visioncats software at a wavelength of 254 and 366 nm using a deuterium and tungsten lamp.

3. RESULTS AND DISCUSSION

Herbo-mineral drugs exhibit inherent variability in their composition due to environmental factors, cultivation practices, harvesting methods, and post-harvest processing. This natural heterogeneity can influence their pharmacological efficacy, bioavailability, and safety. Therefore, establishing a well-defined physicochemical and analytical profile is crucial to ensure standardization, quality control, and therapeutic reliability.

Organoleptic parameters provide a preliminary assessment of the herbal drug's identity and quality based on sensory characteristics. In this study, the PV was observed as a barley shape, black-colored solid with a smooth texture. The odor was identified as pleasant, like honey. The texture was smooth, and the touch was described as hard. These parameters help in verifying the authenticity and consistency of the formulation [Table 2].

Evaluating the physicochemical properties of PV is crucial to assess its stability, purity, and therapeutic potential. The moisture content (loss on drying at 105°C: 4.01% ± 0.12) falls within acceptable limits, reducing the risk of microbial growth and degradation. Controlling moisture is vital, especially in herbo-mineral preparations, to maintain product quality and extend shelf life. The pH of PV is 6.5 ± 0.08, which is close enough to normal tear fluid pH (7.0–7.4). This pH is slightly acidic but still within the acceptable physiological range. It helps to maintain the stability of some drugs that are unstable at neutral or basic pH. Thus, 6.5 ± 0.08 pH is safe and commonly used in ocular preparation. Total ash content (6.19% ± 0.15) reflects the overall mineral and inorganic composition, whereas acid-insoluble ash (0.41% ± 0.02) highlights the level of impurities such as sand or dirt. A low value for acid-insoluble ash suggests high-quality raw ingredients and minimal contamination. Extractive values, such as the alcohol-soluble extractive (19.14% ± 0.10) and water-soluble extractive (41.51% ± 0.25), provide insight into the concentration of bioactive compounds. A high water-soluble extractive value points to the presence of polar compounds such as tannins, flavonoids, and glycosides, which contribute to antioxidant, anti-inflammatory, and anti-cataract effects. Meanwhile, the alcohol-soluble extractive indicates moderately polar substances, such as alkaloids and essential oils, which add to the formulation's therapeutic properties. Tablet characteristics such as friability (0.78% ± 0.01) and hardness (2 ± 0.50 kg/cm²) confirm the mechanical strength of PV. Low friability ensures resistance to breakage during handling, while adequate hardness supports physical stability without hindering disintegration and absorption. Uniform weight (120 ± 4 mg) guarantees consistent dosing and product quality across batches. Variations in weight could lead to inconsistent dosing and affect both efficacy and safety [Table 3].

On qualitative screening of phytochemicals, methanol extracts have shown the presence of alkaloids, tannins, flavonoids, and protein/amino acids while saponins were found in aqueous extracts along with them.

Phytochemical screening of both methanol and aqueous extracts revealed the presence of alkaloids, tannins, flavonoids, and proteins/amino acids, underscoring their potential therapeutic benefits. Alkaloids are known for their antimicrobial and anti-inflammatory effects, whereas tannins and flavonoids contribute antioxidant and astringent properties. In addition, proteins and amino acids play a role in enzymatic functions and tissue regeneration. Interestingly, saponins were found exclusively in the aqueous extract, indicating a possible role in immune system enhancement. Overall, the results suggest notable pharmacological value, with the methanol extract exhibiting antioxidant activity and the aqueous extract demonstrating immune-boosting effects.

On the microscopic evaluation of PV, the diagnostic characteristics of the microscopic analysis of PV showed the presence of microcrystals of calcium oxalate, tracheids of *Haritaki,* and compound starch grains [Figure 1].

The HPTLC study of PV provided valuable insights into its composition, revealing its complex chemical profile. After derivatization, the analysis was conducted at two wavelengths, 254 nm and 366 nm, using different volumes of test samples (6 µL, 8 µL, and 10 µL) to identify and characterize the active components. At 254 nm, for 6 µL, six spots were observed at 0.10, 0.15, 0.33, 0.43, 0.57, and 0.81. Increasing the volume to 8 µL resulted in five spots at 0.11, 0.17, 0.33, 0.44, and 0.82, whereas 10 µL showed six spots at 0.11, 0.19, 0.33, 0.44, 0.53, and 0.82. At 366 nm, for 6 µL, six spots were identified with Rf values of 0.28, 0.34, 0.51, 0.56, 0.68, and 0.89. When the volume increased to 8 µL, four spots appeared at Rf values 0.29, 0.39, 0.52, and 0.55, and for 10 µL, five spots were detected at 0.29, 0.40, 0.52, 0.55, and 0.78 [Figure 2]. The HPTLC profile of PV across different volumes and wavelengths reveals that PV contains a mixture of chemically diverse compounds. Certain major constituents are reliably detectable across conditions. Wavelength and sample volume influence spot detection, indicating the importance of optimizing HPTLC parameters for comprehensive profiling. The analysis supports PV's phytochemical richness and validates HPTLC as a valuable tool for its characterization.

4. CONCLUSION

The investigation of PV highlights its significant antioxidant capacity and potential for treating oxidative stress-related conditions, particularly cataracts. The HPTLC profile of PV reveals that PV contains a mixture of chemically diverse compounds. The analysis supports PV's phytochemical richness and validates HPTLC as a valuable tool for its characterization. Physicochemical testing confirmed the consistency and quality of the formulation, with results showing uniform weight, suitable hardness, acceptable friability, and pH – all of which support its medicinal use. To fully establish PV's effectiveness in cataract management, further *in vivo* and clinical research is necessary. In addition, advanced studies on its pharmacokinetics and mechanisms of action will help facilitate its integration into contemporary ophthalmic treatments targeting oxidative stress-related eye conditions.

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6. AUTHORS' CONTRIBUTIONS

All the authors contributed equally to the design and execution of the article.

7. FUNDING

Nil.

8. ETHICAL APPROVALS

This study does not require ethical approval as it is an experimental study.

9. CONFLICTS OF INTEREST

Nil.

10. DATA AVAILABILITY

This is an original manuscript and all data are available for only review purposes from principal investigators.

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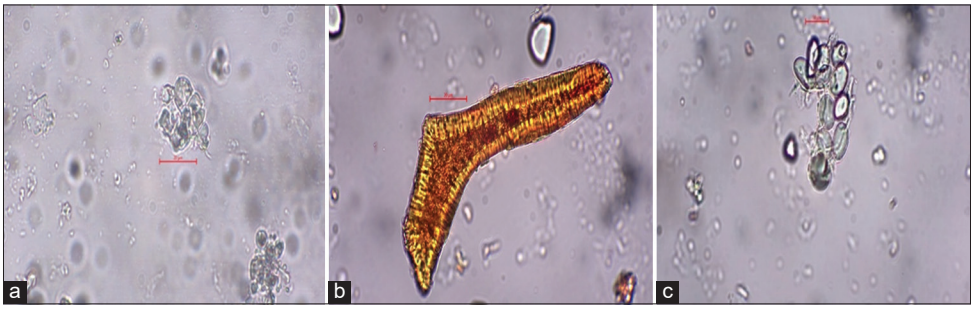

Figure 1: (a) Microcrystals of calcium oxalate, (b) Trachoids of *Haritaki*, (c) compound starch grains

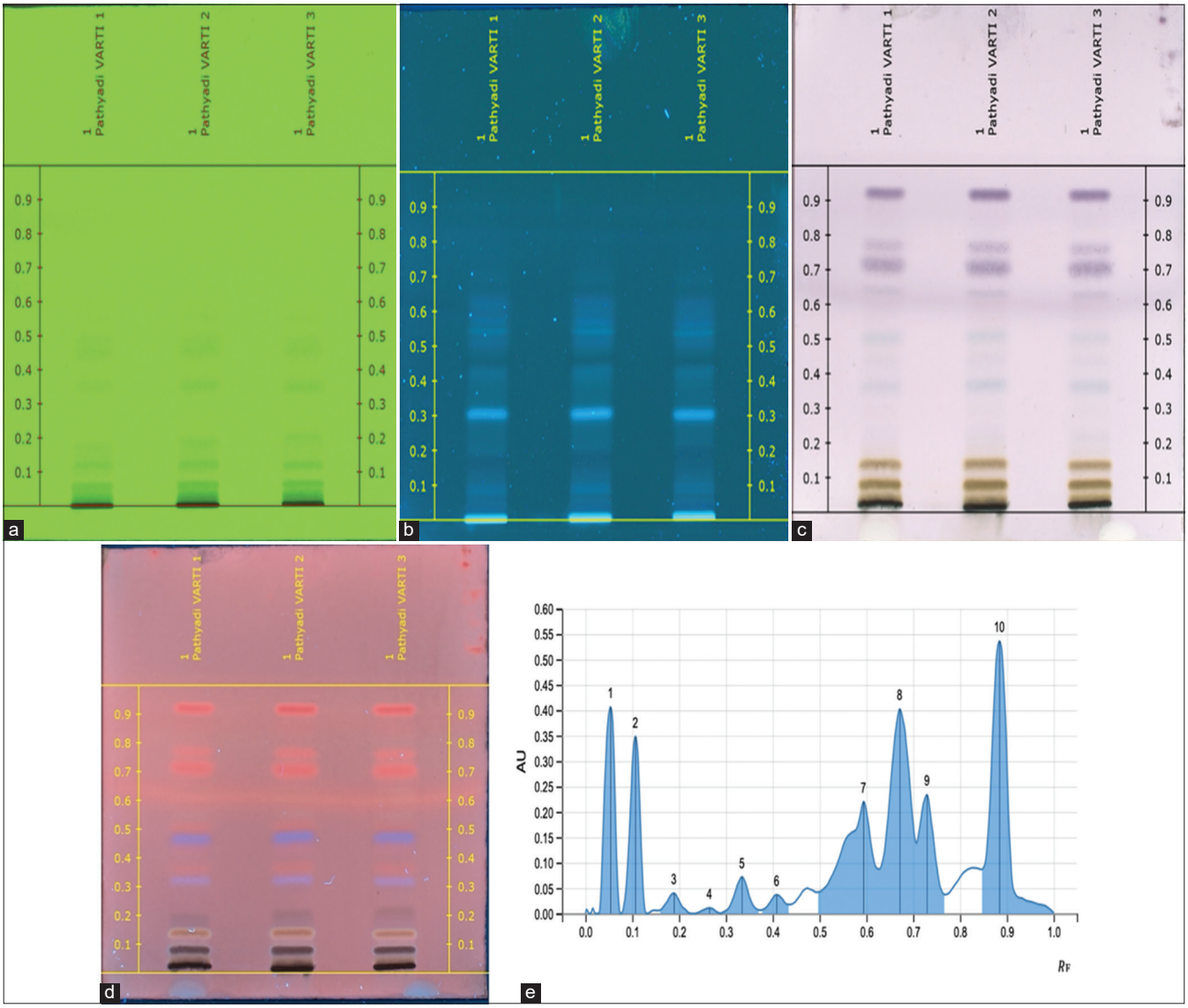

Figure 2: Observed chromatogram and peak at a different wavelength. (a) Under ultraviolet (UV) light-254 nm. (b) Under UV light – 366 nm. (c) After derivatization (R white). (d) After derivatization (R 366). (e) Densitometer @ 540 nm

Table 1: Ingredients of *Pathyadi Varti Anjana*

S. No.	Ingredient	Botanical Name	Part of use	Quantity
1.	*Haritaki*	*Terminalia chebula* Retz.	Pericarp	1 Part
2.	*Yasthimadhu*	*Glycyrrhiza glabra* Linn.	Stem	1 Part
3.	*Tuttha* (copper sulfate)	$CuSo_4$. $5H_2O$	*Shodhita Tuttha*	1 Part
4.	*Shweta Maricha*	*Moringa oleifera* Lam.	Seeds	16 Parts

Table 2: Organoleptic evaluation of PV

S. No.	Parameters	Observations
1.	Appearance	Barley shape and a black colored solid *Varti*
2.	Color	Black
3.	Odor	Pleasant, like honey
4.	Texture	Smooth
5.	Touch	Hard

Table 3: Physicochemical evaluation of PV

S. No.	Parameters	Result (mean±SEM)
1.	Loss of drying at 105°C	4.01%±0.12
2.	pH (5% aqueous solution)	6.5±0.08
3.	Total Ash Value (% w/w)	6.19%±0.15
4.	Acid-insoluble ash (% w/w)	0.41%±0.02
5.	Alcohol-soluble extractive (% w/w)	19.14%±0.10
6.	Water-soluble extractive (% w/w)	41.51%±0.25
7.	Friability	0.78%±0.01
8.	Hardness (kg/cm^2)	2±0.50
9.	Uniformity of weight	120±4 mg
10.	Disintegration time	25 min

Table 4: Phyto-chemical analysis of PV

S. No.	Phytochemicals	Methanol extract	Aqueous extracts
1.	Alkaloids	+	+
2.	Tannins	+	+
3.	Flavonoids	+	+
4.	Protein/Amino acids	+	+
5.	Saponins	-	+

(+): Present, (-): Absent