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Analytical Evaluation and Standardization of Vyoshadhi Varti Anjana in Kaphaja Timira: An Ayurvedic Formulation

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Abstract:
Vyoshadhi Varti Anjana is an ayurvedic topical formulation explained in Bhaisajya Rathnavali Netraroga Adhikara under the context of Timira Chikitsa. In the present era, despite the extensive clinical usage of traditional Ayurvedic formulations, there is a growing need for their scientific validation and standardization. The present study was carried out on analytical evaluation of Vyoshadhi Varti Anjana to establish its physicochemical and phytochemical parameters in accordance with standard guidelines. **Materials and Methods:** Vyoshadhi Varti Anjana was prepared as per classical Ayurvedic procedures. The formulation was subjected to organoleptic evaluation and physicochemical analysis including parameters such as loss on drying, ash values, pH, and extractive values. Preliminary phytochemical screening was also performed to identify the presence of major phytoconstituents. **Results:** The analytical evaluation revealed acceptable physicochemical values within permissible limits, indicating good quality and stability of the formulation. Phytochemical screening confirmed the presence of bioactive constituents supporting its therapeutic values. The findings of the present study provide preliminary scientific data for the standardization of Vyoshadhi Varti Anjana. The data helps in establishing quality control parameters and supports the safe and effective use of this classical Ayurvedic topical ophthalmic formulation Vyoshadhi Varti Anjana.

Keywords: Vyoshadhi Varti Anjana, Kaphaja Timira, immature cortical cataract, analytical study.

Introduction:

Vyoshadivarti Anjana is mentioned in Bhaisajya Rathnavali in Netra Roga Adhikara [1] containing Pippali, Maricha, Shunti, Utpala, Kusta, Rasanjana is Tikshna, Ushna and Lekhaniya in nature. The formulation is specifically mentioned in context of Kaphaja Timira which is one among the Dristigataroga where the person experiences Avyaktha Darshana, Snigdha Darshana and Jalaka Darshana [2]. Considering the clinical features and stages of cataract Senile Immature cataract can be correlated to Kaphaja Timira. Authentic literatures prescribed several preventive and curative measures for the management of Timira. Immature stage of cortical cataract can be understood as increase of Kleda bhava of Kapha Dosha when it is lodged in first two Patalas Tejojala Ashrita and Mamsashrita. Treatment principles explained for Timira in classics are Ghritapana, Nasya, Anjana, Siravyadhana. Among the Kriya Kalpa Anjana has given importance in the management of Dristigata Rogas. Anjana is a medicinal preparation applied on the lower palpebral conjunctiva which can reach the deeper layers of the eye and helps in scraping out the Doshas

[3]. Especially Lekhana Anjana has been emphasized in Kaphaja Timira as the Samprapthi Vighatana needs drugs with Ushna, Tikshna, Sukshma Gunas [4]. A need for a non-surgical treatment like local application is necessary which would be more accessible and non-invasive. A drug that treats or manages the growth of cataract will not only improve the quality of life but also reduce the public health burden of vision impairment as senile cataracts are the leading cause for blindness.

Therefore, present study was carried out on analytical evaluation of Vyoshadhi Varti Anjana to establish its physicochemical and phytochemical parameters in accordance with standard guidelines. To establish quality control parameters and supports the safe and effective use of this classical Ayurvedic topical ophthalmic formulation Vyoshadhi Varti Anjana in Kaphaja Timira.

Methodology

Preparation of the medicine [5]:

Fine powder of the above drugs was taken mixed together to get a homogenous mixture. Sufficient quantity of water added and Bhavana was done. Bhavana procedure was continued until the Kalka attained Subhavitha Lakshanas. The Subhavitha Kalka was rolled into Varti form and were dried under shade. Once the Vartis were properly dried it was stored in an air tight container.

Loss on drying at 105°C

10 g of sample (Varti) was placed in tarred evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash

2 g of sample (Varti) was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash: To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

Alcohol soluble extractive

Weigh accurately 4 g of the sample (Varti) in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive: Weigh accurately 4 g of the sample (Varti) in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to

dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

Determination of pH

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1gm of sample (Varti) was taken and was dissolved in 100ml of distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. for 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

Hardness test

Five Varti's were taken and tested for hardness. The lower plunger was placed in contact with the tablet. The upper plunger was then forced against a spring by turning a threaded bolt until the tablet fractures. The force of fracture was recorded.

Friability

10 Vyoshadi Varti Anjana were weighed after being dusted. Varti's were then placed in the drum of friabilator and rotated for 100 times. Varti were taken out after rotation then dusted and precisely weighed. The difference in weight was measured, which indicates friability.

Interpretation of Physicochemical Parameters of Vyoshadi Varti Anjana:

Parameters	Results	Interpretation
Loss on drying	0.15±.02	This value indicates low moisture/volatile content which is important for stability and prevents microbial growth.
Total ash	8.31±0.21	It is within normal limits for herbal drugs indicating both organic and inorganic ash
Acid insoluble ash	0.0±0.0	Indicates there was no insoluble inorganic matter or adulteration in that particular sample.
Alcohol soluble extractive value	24.03±0.01	Indicates presence of alkaloids, volatile substances
Water soluble extractive value	44.81±0.01	Indicates the presence of constituents soluble in water such as tannins, glycosides.
pH	6.22	Weakly acid but near neutral. Drug remains stable at this pH.
Hardness (Kg/cm^2)	5.0	Within the normal acceptable range, has adequate mechanical strength for easy handling and transport.
Friability (%)	0.1	Indicates the Varti have excellent mechanical strength and can withstand handling, packaging, and transport without significant breakage
These parameters collectively confirm that the formulation is pharmaceutically stable, mild irritant, and safe for administration.		

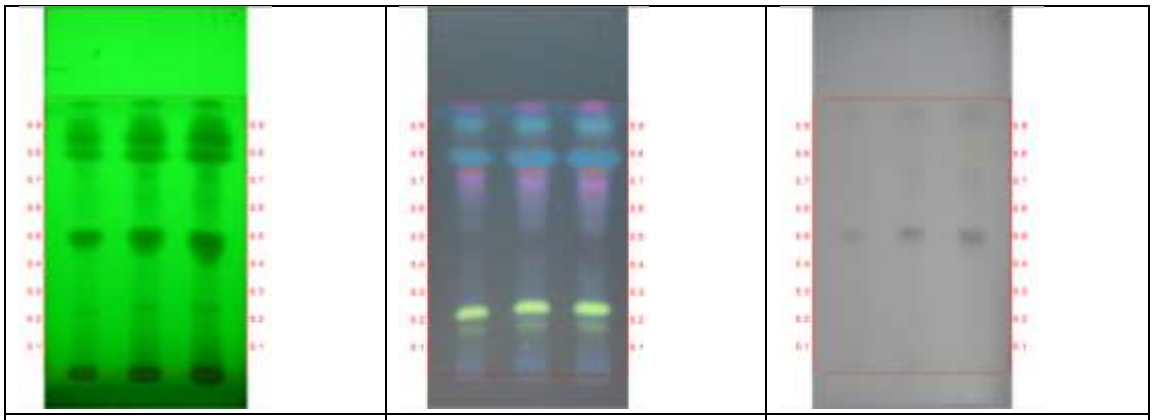
High Performance Thin Layer Chromatography (HPTLC):

One gram of powdered sample of Vyoshadi Varti Anjana was dissolved in 10 ml ethanol and was warmed, filtered. 3, 6 and 9µl of the above filtrate was applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate: Formic acid: Methanol (3.0: 3.0: 0.8: 0.2). The developed plates were visualized in short UV, Long UV and then derivatized with Anisaldehyde Sulphuric Acid (ASA) reagent and scanned under UV 254nm, 366nm and 620nm. Rf, Colour of the spots and densitometric scan were recorded.

Table 1. Results of physicochemical parameters of Vyoshadi Varti Anjana

Parameters	**Results n=3 % w/wAvg ± SD**
	Vyoshadi Varti Anjana
Loss on drying	0.15±.02
Total ash	8.31±0.21
Acid insoluble ash	0.0±0.0
Water soluble ash	4.06±0.02
Alcohol soluble extractive value	24.03±0.01
Water soluble extractive value	44.81±0.01
pH	6.22
Hardness (Kg/cm^2)	5.0
Friability (%)	0.1

Figure 1. HPTLC photo documentation of ethanolic fraction of Vyoshadi Varti Anjana



Short UV	**Long UV**	**Post derivatization**

track 1 - Vyoshadi Varti Anjana – 3µl track 2 - Vyoshadi Varti Anjana – 6µl track 3 - Vyoshadi Varti Anjana – 9µl Solvent system – Toluene: Ethyl acetate: Formic acid: Methanol (3.0: 3.0: 0.8: 0.2) 254nm, ASA, R_f - 0.91

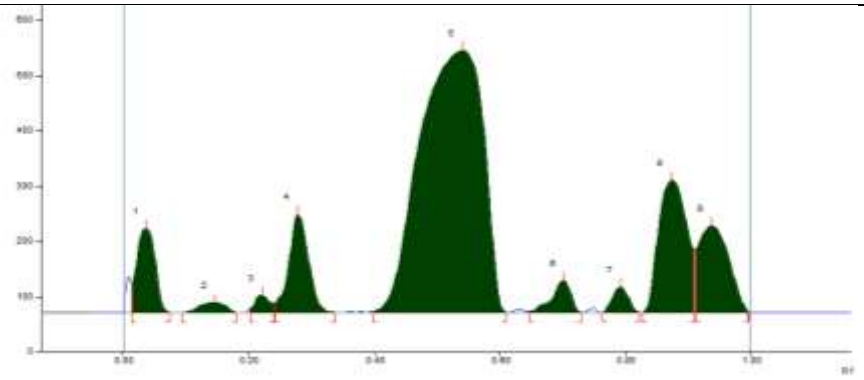
Table 2: R_f values of samples

At 254nm	**At 366nm**	**Post derivatization**
	0.17 (F. green)	-
0.24 (Green)	0.24 (F. green)	-
0.49 (Green)	-	0.49 (Purple)

0.64 (Green)	0.63 (F. purple)	-
-	0.66 (F. red)	-
-	0.69 (F. purple)	-
0.72 (Green)	0.71 (F. red)	0.73 (Purple)
0.79 (Green)	0.78 (F. blue)	-
0.85 (Green)	-	-
0.91 (Green)	0.90 (F. blue)	-
-	-	0.94 (Purple)

***F – fluorescent; L –light; D – dark**

Figure 2. Densitometric scan at 254nm

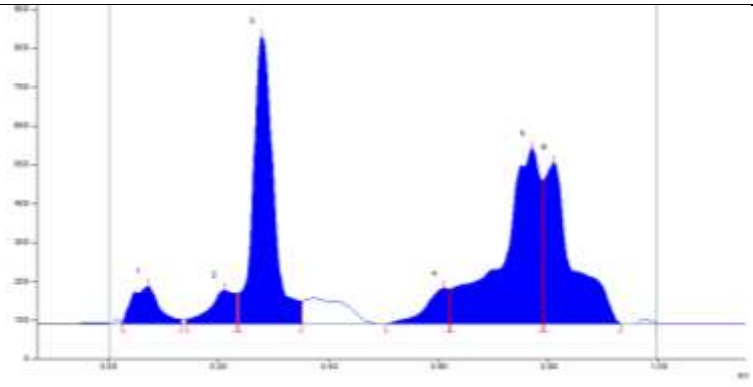


Track 3, ID: Vyoshadi varti anjana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	53.4 AU	0.04 Rf	152.9 AU	11.27 %	0.07 Rf	0.4 AU	3223.7 AU	5.52 %
2	0.10 Rf	0.1 AU	0.15 Rf	18.6 AU	1.37 %	0.18 Rf	0.2 AU	561.0 AU	0.96 %
3	0.20 Rf	7.8 AU	0.22 Rf	32.0 AU	2.36 %	0.24 Rf	17.0 AU	535.3 AU	0.92 %
4	0.24 Rf	17.5 AU	0.28 Rf	178.2 AU	13.13 %	0.34 Rf	0.2 AU	4015.0 AU	6.88 %
5	0.40 Rf	1.8 AU	0.54 Rf	474.0 AU	34.94 %	0.61 Rf	0.1 AU	34608.7 AU	59.31 %
6	0.65 Rf	2.6 AU	0.70 Rf	58.4 AU	4.31 %	0.73 Rf	0.2 AU	1277.6 AU	2.19 %
7	0.76 Rf	0.1 AU	0.79 Rf	46.0 AU	3.39 %	0.82 Rf	0.4 AU	853.5 AU	1.46 %
8	0.83 Rf	0.2 AU	0.87 Rf	239.6 AU	17.66 %	0.91 Rf	15.0 AU	7797.8 AU	13.36 %
9	0.91 Rf	115.2 AU	0.94 Rf	157.0 AU	11.57 %	1.00 Rf	2.6 AU	5483.3 AU	9.40 %

Vyoshadi varti anjana, R_f - 0.91 (9.40%)

Figure 3. Densitometric scan at 366nm

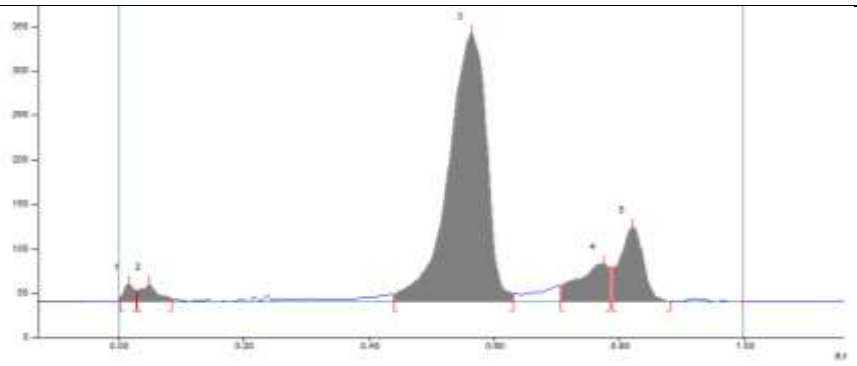


Track 3, ID: Vyoshadi varti anjana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	4.7 AU	0.07 Rf	96.3 AU	5.13 %	0.13 Rf	11.2 AU	3357.0 AU	5.05 %
2	0.14 Rf	11.9 AU	0.21 Rf	86.2 AU	4.59 %	0.24 Rf	77.7 AU	2864.1 AU	4.31 %
3	0.24 Rf	77.9 AU	0.28 Rf	739.0 AU	39.35 %	0.35 Rf	58.2 AU	19612.8 AU	29.52 %
4	0.50 Rf	0.6 AU	0.61 Rf	90.9 AU	4.84 %	0.62 Rf	89.5 AU	2732.3 AU	4.11 %
5	0.62 Rf	89.7 AU	0.77 Rf	451.2 AU	24.03 %	0.79 Rf	68.7 AU	22448.3 AU	33.79 %
6	0.79 Rf	369.1 AU	0.81 Rf	414.3 AU	22.06 %	0.93 Rf	0.3 AU	15416.7 AU	23.21 %

Vyoshadi varti anjana

Figure 4. Densitometric scan at 620nm (after derivatization with ASA)



Track 3, ID: Vyoshadi varti anjana

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.5 AU	0.02 Rf	19.4 AU	4.16 %	0.03 Rf	11.7 AU	239.5 AU	1.36 %
2	0.03 Rf	12.0 AU	0.05 Rf	19.2 AU	4.11 %	0.09 Rf	3.1 AU	386.6 AU	2.20 %
3	0.44 Rf	8.1 AU	0.56 Rf	302.1 AU	64.65 %	0.63 Rf	8.9 AU	13180.8 AU	74.92 %
4	0.71 Rf	17.4 AU	0.78 Rf	42.8 AU	9.16 %	0.79 Rf	37.7 AU	1543.8 AU	8.78 %
5	0.79 Rf	38.4 AU	0.82 Rf	83.7 AU	17.92 %	0.88 Rf	0.4 AU	2242.6 AU	12.75 %

Vyoshadi varti anjana

Interpretation of HPTLC Profile (High Performance Thin Layer Chromatography):

High Performance Thin Layer Chromatography (HPTLC) was performed to evaluate the chemical fingerprint of the ethanolic fraction of Vyoshadi Varti Anjana. This method helps in qualitative identification, profiling, and standardization of the formulation by visualizing the presence of phytoconstituents through chromatographic separation.

Track	Sample volume	Observation method	Rf Value	Colour of Spot	Inferred Constituents
1	3 µl	Short UV (254 nm)	0.24 (Green)	Green	Possibly alkaloids/flavonoids or aromatic compounds
2	6 µl	Long UV (366 nm)	0.24	Green	Indicative flavonoids, coumarins, terpenoids
			0.63	Purple	Possibly anthraquinones and phenolic compounds
			0.71	F. red	Possibly quinones, alkaloids, or coloured flavonoids

3	9 µl	Post derivatisation	0.68	Purple	Reacting constituents, possibly phenols, aldehydes, steroids.

Densitometric Scanning:

Densitogram at 254 nm (Short UV): indicates the presence of chemically active principles like flavonoids or polyphenolic derivatives, known for their antioxidant and anti-inflammatory properties.

Densitogram at 366 nm (Long UV): Confirms the presence of phenolic and alkaloid phytochemicals, in the preparation.

Densitogram at 620 nm (After Derivatisation with Vanillin Sulphuric Acid): Indicates the presence of chemically reactive constituents which become more visible only after chemical derivatisation—this adds specificity and supports the compound class**.**

Discussion:

In this study drug, the analytical evaluation indicates that nearly 50% of the drugs are hydrophilic. These drugs penetrate the conjunctiva, following which the sclera exhibits greater permeability, facilitating the drugs access to other structures, including the ciliary body, iris, choroid, and lens. Hydrophilic constituents such as flavonoids, alkaloids, glycosides in the Anjana get absorbed through the conjunctiva by paracellular pathway. This ocular absorption may depend on the passive diffusion, carrier mediated transport (facilitated diffusion and active transport) and endocytosis. pH of the Varti Anjana is slightly acidic (weak acid) so it is unionized form which have better penetration through corneal epithelium. With the above explanation it is understood that it can reach all the anterior segment parts of the eye including lens. As the cortex is the most superficial fibres the drug availability is more when compared to deeper parts of the lens.

The presence of distinct bands at different Rf values and their specific colours under UV and visible light confirm the multicomponent nature of the Varti. Repeated sample application (3, 6, and 9 µl) ensures dose-dependent consistency in band intensity, supporting reproducibility. The Rf values serve as reference fingerprints for future quality control and batch-to-batch standardization of this formulation. The derivatisation further confirms chemical class identification which is crucial for pharmacological correlation.

Conclusion:

The HPTLC profile of Vyoshadi Varti Anjana demonstrates the presence of multiple bioactive phytoconstituents. The developed chromatogram can be used as a standard fingerprint marker for this formulation. The clear and reproducible bands at Rf 0.24, 0.63, and 0.71 support the formulation's authenticity, stability, and quality. This analytical approach strengthens the scientific validation of classical Ayurvedic preparations in alignment with pharmacognostical standards.

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