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Safety Standardization of *Vishtinduk Vati*: Quantitative HPTLC Estimation and Spectroscopic Fingerprinting of Toxic Alkaloids

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KEYWORDS

Vishtinduk Vati, *Strychnos nux-vomica*, strychnine, brucine, HPTLC

ABSTRACT:

Introduction: Strychnos nux-vomica is a potent therapeutic agent in Ayurveda, used in Ayurvedic formulations to treat a variety of ailments, including rheumatism, digestive problems, skin concerns, and neurological disorders like paralysis and sciatica. It has different phytoconstituents such as strychnine, brucine, loganin etc. Vishtinduk Vati is an excellent nerve tonic and cardiac stimulant. However, when taken in large doses, strychnine and brucine can be toxic and induce severe convulsions of the muscles. As a result, further incentives are required to do comprehensive investigations on the safety and effectiveness of Vishtinduk Vati. Phytochemical fingerprinting has become a prominent technique for assessing the effectiveness and authenticity of Ayurvedic medicines. Researchers may generate a distinct "chemical profile" for herbal compositions via the use of spectroscopic tools and chromatographic methods.

Objectives: This study aims to establish chemical characteristics using spectroscopic instruments and chromatographic method.

Methods: A UV-Vis spectrophotometric analysis was performed to analyze the characteristic absorption peaks of the phytochemicals in Vishtinduk Vati. FTIR spectrum of Vishtinduk Vati generates a unique spectral fingerprint and provides information about the molecular vibrations and functional groups. Strychnine and brucine in Vishtinduk Vati were simultaneously and quantitatively analyzed by High Performance Thin-Layer Chromatography.

Results: The UV-Vis spectrophotometric study revealed three absorbance peaks in the UV-Vis spectrum, while the solid-state IR (KBr) spectra showed characteristics stretching N-H group, C-H (Ar) group and C=O group. Quantitative determination via HPTLC and subsequent linear regression analysis revealed the contents of strychnine and brucine to be 0.026 ± 0.0095 g/100 g and 0.0702 ± 0.090 g/100 g, respectively.

Conclusions: Strychnine and brucine in Vishtinduk Vati extracts were analyzed accurately, reliably, and precisely using the HPTLC method. This technique was used to determine the amounts of strychnine and brucine and in the extracts. Compared to raw Strychnos nux-vomica seeds, the Vishtinduk Vati extract exhibited lower levels of these alkaloids.

1. Introduction

Ayurveda is a traditional medical practice that began in the world over 5,000 years ago. It is founded on the principle that health and well-being depend on a fragile equilibrium among the mind, body, and spirit.[1–3]The philosophy of ayurveda is distinctive, being that it provides the prospect for a healthy, peaceful, and prolonged existence.[4]Ayurvedic formulations are traditional medicines derived from ancient Indian literature and practices. Instead than only treating symptoms, these aim to address the underlying cause of health problems.[5] Ayurvedic formulations are prepared from plants (Kasthausadhi) such as Asava, Aristra, Avleha, Churna, Taila, and metals and minerals(Rasausadhi) like Bhasma, Lauha, Kapibadkva, and Rasayana.[6,7] Ayurvedic herbs contain a variety of phytochemicals.These phytochemicals are crucial to therapeutic functions.[8] In contrast the majority of plant-based medications are harmless, only a few are hazardous to human health.[9] Traditional Ayurvedic texts prohibit the use of herbs whose properties are not fully understood. Even poisons may have therapeutic

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effects when used properly, and the texts warn against misusing well-known herbs, highlighting that even the most exquisite medicines can be lethal when misused.[10] In Ayurveda, toxic plants are classified as either visa (poison) or upaviṣa (harmful but not destructive to human health).[11] The process of detoxification or purification of harmful substances is known as "Śodhana" in ayurveda, and hence, such herbal treatments must be purified before being used in medicine.[12]The Sodhana encompasses the purifying and detoxification of both physical and chemical impurities, and also addressing the reduction of side effects and enhancement of the potency and therapeutic effectiveness of the cleansed substances.[13,14] Some examples of poisonous herbs that are used in ayurvedic formulations are *Strychnos nux-vomica* (Vishtinduk Vati), *Aconitum ferox* (VatsanabhaTaila), and *Nerium oleander* (Arka Ghrita).

Strychnos nux-vomica Linn., from the *Loganiaceae* family, often known as *Kuchla*, is a medium-sized tree that is extensively found in India's deciduous forests in the eastern and southern regions of the nation.[15] Despite its toxic effects, *Strychnos nux-vomica* is still used in the medical field after purification (detoxification).[16] It has strychnine (1.25-1.5%) and brucine (1.7%) as primary components.[17] The seeds include minor alkaloids such as protostrychnine, vomicine, n-oxystrychnine, pseudostrychnine, isostrychnine, chlorogenic acid, and a glycoside.[18] *Strychnos nux vomica* seeds are used to cure a variety of conditions, such as anaemia, bronchial asthma, bronchitis, diabetes, skin conditions, paralysis, mental disorders, chicken pox fever, eczema, and rheumatism.[19,20] It is a significant treatment for amytonia and relaxation of the stomach and intestines, as well as related illnesses. Ayurvedic texts have reported its use as an analgesic, stimulant, and for treating urinary incontinence resulting from a weakened or paralyzed sphincter. It serves as an anxiolytic and erotic.[21–23]

On the other hand, strychnine and brucine, when taken in large doses, may be very toxic and can induce severe convulsions of the muscles.[24,25] Strychnine increases the heartbeat, increases blood pressure, and can be used as a circulatory system tonic in cases of cardiac failure.[26] According to reports, the likely lethal oral dose is 60 to 90 mg for adults or 1.5 to 2 mg/kg.[27,28] Brucine is typically used as an analgesic and anti-inflammatory medication to treat arthritis and traumatic pain.[29] Recent studies on a variety of tumours showed that brucine had remarkable anti-tumor effects.[30] According to literature survey, the likely fatal oral dosage is 50.10 mg/kg, or 1g for adults.[31,32]

Strychnos nux-vomica is an ingredient in many classic ayurvedic formulations. More than 60 formulations, including *Strychnos nux-vomica*, such as *Agnitundi Rasa, Navjivan Rasa, and Shoolnirmoolan Rasa*, are documented in the literature of Indian systems of medicine (ISM).[17]

Vishtinduk Vati is an ayurvedic medicine referenced in *Ras Tantra Saraand Sidha Prayoga Samgraha*.[33] The primary ingredient of V*ishtinduk vati* is *Strychnos nux-vomica* (*Kuchla)*. According to Ayurvedic texts, *Strychnos nux-vomica* safely incorporated into *Vishtinduk Vati* after undergoing proper processing or purification, which will reduce or eliminate their adverse or poisonous effects. *Vishtinduk vati* functions as both a nervine and a cardiac stimulant. It is helpful in alleviating localised paralysis, facial paralysis, and neuralgia. It has digestive, purgative, and analgesic properties.[34]

In previous work, Namba *et al* reported that the heat treatment to the seeds of *Strychnos nux-vomica* decreases the concentrations of strychnine and brucine.[35] Katiyar *et al* reported that the shodhan treatment change in phytochemicals and reverses the pharmacological profile of the *Strychnos nux-vomica*.[16] Zhang *et al* isolated fifteen compounds from the processed seeds of *Strychnos nux-vomica*.[36] Mitra *et al* reported that the toxic alkaloids strychnine and brucine content reduced after purification of *Strychnos nux-vomica* seed.[37] Patel *et al* reported phytoconstituent analysis and HPTLC analysis of *Strychnos nux-vomica* seed extracts.[38] Shukla *et al* reported purification process of *Strychnos nux vomica* seed affects the physical and chemical properties.[39] Patil *et al* reported isolation and identification of brucine from *Vishtindukdi vati*.[40]

2. Objectives

There are limited studies on the *Shodhana* element of *Strychnos nux-vomica* seed, according to a review of several research articles, ayurvedic textbooks, and search engines. Numerous studies have reported pharmacological changes resulting from the sodhana process of *Strychnos nux-vomica* seeds highlighting the need for further research on phytochemicals and the impact of compositional difference on biological activity. Therefore, the goal of the current study is to use the HPTLC method to measure the amount of strychnine and brucine from *Vishtinduk Vati*.

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3. Methods

Reagents and Chemicals

Vishtinduk Vati was procured from a local ayurvedic pharmacy. Various analytical-grade reference standards and solvents were obtained from Merck India Ltd., Mumbai, India. All markers used were procured from Yucca Enterprises, Mumbai, India.

Equipment

For the extraction, the Ragatech Microwave was employed. UV–Visible spectrophotometric analysis was carried out using a Jasco V-630 spectrophotometer, while Fourier Transform Infrared (FTIR) analysis was conducted with a Shimadzu FTIR-8400S instrument. WINCATS software and the CAMAG TLC Scanner 3 software were used for densitometric scanning of the TLC plate.

Sample preparation

Weigh 20 *Vishtinduk Vati* samples, calculate the average weight, and grind them into a fine powder. In a round-bottom flask (RBF), add 10 mL of methanol and 1 g of Vati powder, microwave at 280 W for 10 minutes, and then filter through a 0.45 µm filter membrane. Concentrate the extract using a rotary evaporator, and store the residue in a sealed container for further studies.

Preparation of Standard stock solution

Standard stock solutions of strychnine and brucine (1000 µg mL^{-1}) were prepared by accurately weighing 10 mg of each compound and making up the volume to 10 mL with methanol. From these, working standard solutions (100 µg mL^{-1}) were obtained by transferring 1.0 mL of the respective stock solution and adjusting the final volume with methanol.

Experimental

Physical parameter evaluation

The physical properties of *Vishtinduk Vati* including total ash value, loss on drying, acid-insoluble ash values, alcohol soluble extractive value, water-soluble extractive value etc. were analyzed as per pharmacopoeial methods.

UV-Vis spectrophotometric analysis

The UV-Visible absorption spectrum of *Vishtinduk Vati* extract was recorded in the wavelength range of 200–400 nm using a Jasco UV-Vis spectrophotometer.

FTIR spectral analysis

The FTIR spectra of *Vishtinduk Vati* extract in the spectral range of 4000 to 400 cm^{-1} were recorded using diffuse reflectance spectroscopy (DRS).

HPTLC Analysis

Preparation of test solution

Dissolve 10 mg of *Vishtinduk Vati* extract in 10 mL of methanol to prepare a test solution. After sonication for 10 minutes, filter the mixture using Whatman 41 filter paper. Take 1.0 mL of the obtained sample stock solution and dilute it with 10 mL of methanol to obtain a final sample stock solution with a concentration of 100 µg mL^{-1}.

Chromatographic conditions

Chromatographic study of *Vishtinduk Vati* was performed using HPTLC system. A 100 µL sample syringe and a sample applicator were used to apply bands of markers and *Vishtinduk Vati* extract onto prewashed aluminum plates (10 × 10 cm, Merck, Darmstadt, Germany) coated with silica gel 60 F254.The mobile phase, toluene: ethyl acetate: methanol: formic acid (1.5:2:0.2:0.8, v/v/v/v), was used to develop the TLC plates in a glass chamber. The mobile phase was allowed to saturate the chamber for 20 min at room temperature. The developed TLC plate was analyzed at a wavelength of 254 nm using a CAMAG TLC scanner, and the data was processed using WINCATS software.

Method Validation

The proposed methods for the determination of strychnine and brucine were validated in accordance with the guidelines recommended by the International Council for Harmonization (ICH).[41]

Specificity

The specificity of the method was evaluated by spotting the reference standard and the *Vishtinduk Vati* sample onto high-performance thin-layer chromatography (HPTLC) plates, respectively. The peak purity of strychnine and brucine was confirmed by comparing the spectral characteristics of the onset, middle, and end regions of each chromatographic peak.

Linearity

Linearity was evaluated within a concentration range of 400–900 ng/band for strychnine and brucine, respectively. Each concentration level was measured three times. Peak area versus concentration was plotted to establish a calibration curve, and the slope, intercept,

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and correlation coefficient were calculated using linear regression method.[42]

Limit of detection (LOD) and limit of quantification (LOQ)

Linear regression analysis was employed to determine the standard deviation of the peak regions for each marker. These values were subsequently used to establish the Limits of Detection (LOD) and Limits of Quantification (LOQ).[43]

Precision

Intraday and interday studies were carried out to assess the precision of the method.[44] The % Relative Standard Deviation was determined by applying six replicate bands of strychnine and brucine solutions on the same day and on three consecutive days, respectively. (Table 2.)

Robustness

The robustness of the method was assessed by intentionally changing experimental parameters such as mobile phase composition and chamber saturation time, and the degree to which the process maintained consistency was examined.[45]

Accuracy

Accuracy was evaluated using the standard addition method for sample analysis. Strychnine and brucine were spiked to 50, 100, and 150% of a pre-quantified extract of *Vishtinduk Vati*, followed by measurements of their responses (peak area), which allowed for the calculation of the percentage recovery.

The accuracy was calculated from the following equation: [(spiked concentration - mean concentration) / spiked concentration] × 100.[46]

Quantification

Vishtinduk Vati extract was spotted onto a silica gel 60 F_{254} coated aluminum plate. After development in an optimized mobile phase, the thin-layer plates were scanned at a wavelength of 254 nm, to record peak areas. Quantification was subsequently performed via a linear regression method.

Statistical Analysis

All statistical analysis were performed using Microsoft Excel.

4. Results

Physical parameters of marketed *Vishtinduk Vati* were evaluated. The loss on drying of *Vishtinduk Vati* was found to be 3.86% w/w, low moisture content ensuring the stability and shelf life of the vati. The total ash value of *Vishtinduk Vati* was found to be 19.36 % w/w, indicating the presence of a significant amount of inorganic constituents in the vati.This value is essential for determining the quality and purity as well as its mineral content. The acid-insoluble ash values of *Vishtinduk Vati* was found to be 4.51%, highlights the presence of inorganic materials that are insoluble in acid. Measuring acid-insoluble ash is a critical step in evaluating the quality of vati, as it helps to identify impurities and adulterated matter. *Vishtinduk Vati's* water-soluble extractive value was found to be 32%, suggesting that the formulation contains a significant amount of water-soluble components. The alcohol-soluble extractive value of *Vishtinduk Vati* was found to be 13%, indicates the presence of active constituents in the vati that are soluble in alcohol. The result reported in Table 1.

Table 1:- Physical parameters of marketed *Vishtinduk Vati*

Parameter	**Result**
Loss on drying (110 ^{0} C)	3.86 ± 0.085 % w/w
Total ash value	19.36 ± 0.20 % w/w
Acid-insoluble ash values	4.51 ± 0.055 % w/w
Water-soluble extractive value	32 ± 0.46 % w/w
Alcohol soluble extractive value	13 ± 0.15 % w/w
Hardness	7.25±0.11 Kg/cm^2
Disintegration time	24.25±0.30 min
Friability	1.06±0.018 % w/w

UV-Vis spectrophotometric analysis

The UV-Vis absorption spectrum of *Vishtinduk Vati* extract (Fig. 1) was recorded in the wavelength range of 200–400 nm using a Jasco UV-Vis spectrophotometer. Three separate absorption maxima were observed at wavelengths of 251 nm, 298 nm, and 340.5 nm, respectively.

FT-IR spectrophotometric analysis

The FTIR spectrum of the *Vishtinduk Vati* extract (Fig. 2) revealed an N-H stretching band between 3350 and 3360 cm^{-1}. The presence of the Ar (C-H) group was confirmed by stretching observed at 3070–3180 cm^{-1},

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while the C-H group was identified through stretching at 2962 cm^{-1}. A stretching band at 1643 cm^{-1} identified the C=O group, whereas the C=C group was indicated by stretching at 1512 & 1419 cm^{-1}. Additionally, the presence of the C-N group was verified by stretching in the range of 1250–1310 cm^{-1}.[47]

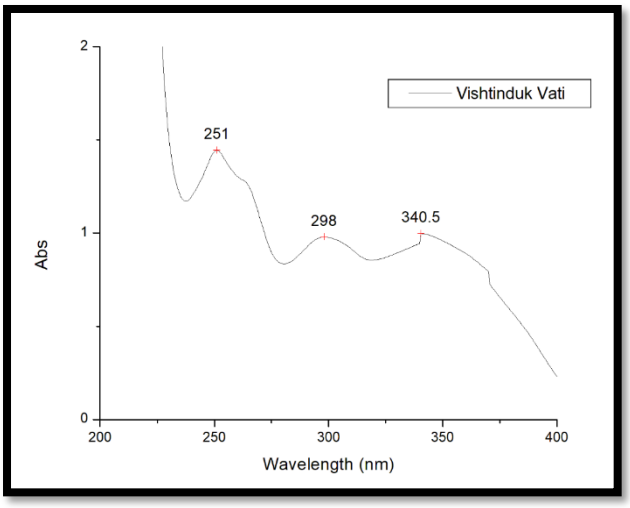

Fig 1. UV-Visible Spectrum of *Vishtinduk Vati* Extract

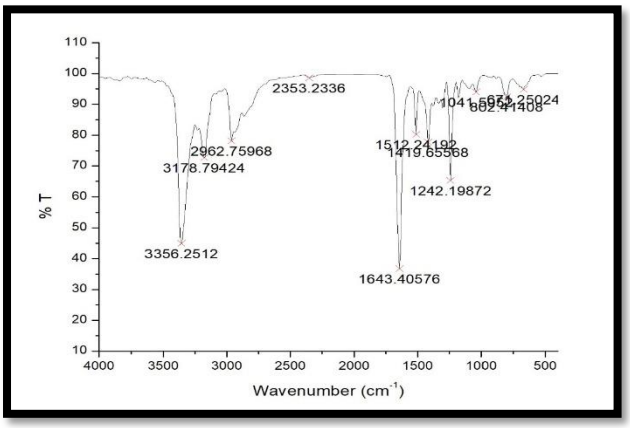

Fig 2. Fourier Transform Infrared (FTIR) Spectrum of *Vishtinduk Vati* Extract

HPTLC analysis

The *Strychnos nux-vomica* extract was spotted onto a pre-coated silica gel thin-layer chromatography plate. A mobile phase of toluene, ethyl acetate, methanol, and formic acid (volume ratio 3:4:0.4:1.6) was used for the separation of phytochemicals. At 254 nm, strychnine and brucine appeared as clear spots, with Rf values of 0.36±0.002 and 0.30±0.002, respectively ((Fig. 3).

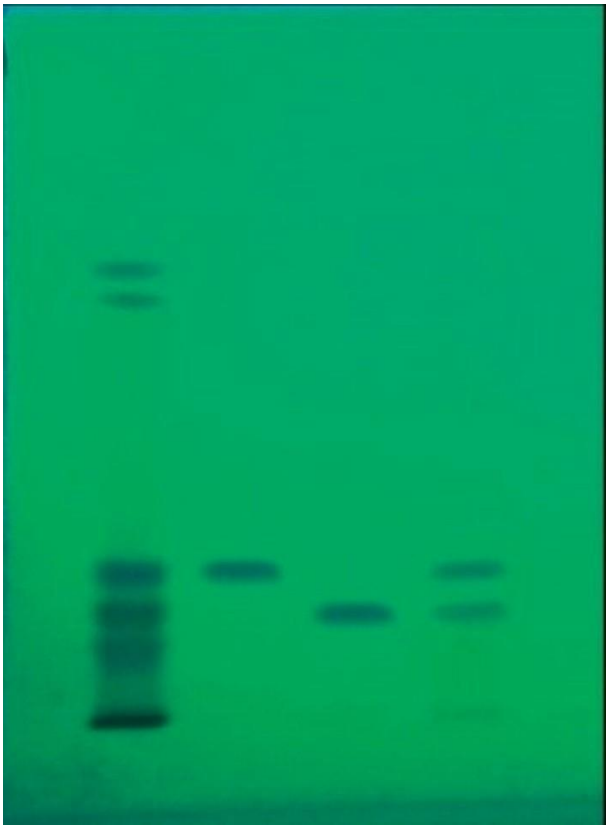

Fig 3. TLC plate of *Vishtinduk Vati* Extract

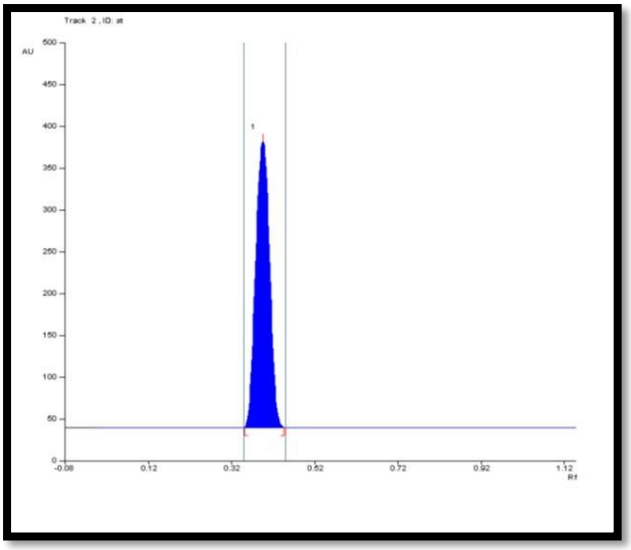

Fig 4. Chromatogram of Strychnine

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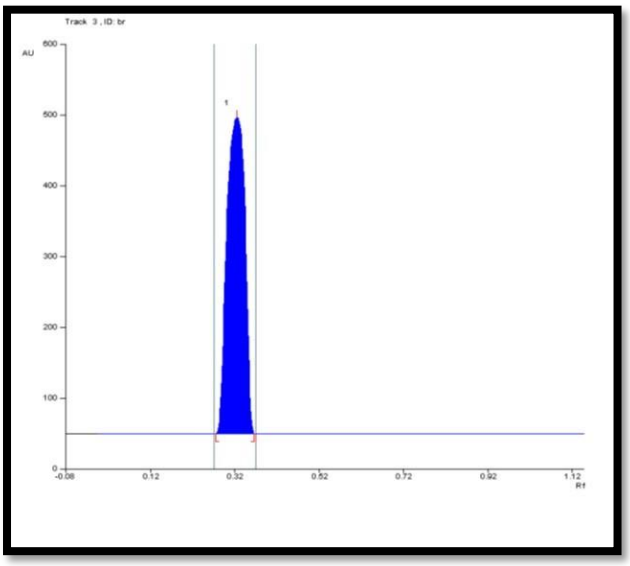

Fig 5. Chromatogram of Brucine

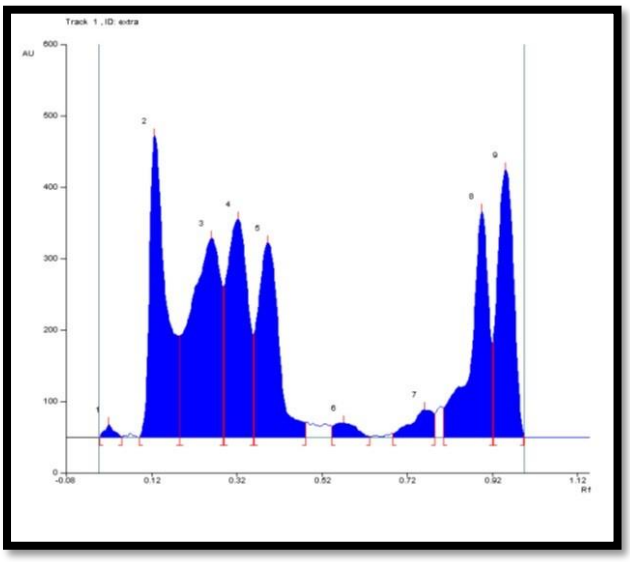

Fig 6. Chromatogram of *Vishtinduk Vati* Extract

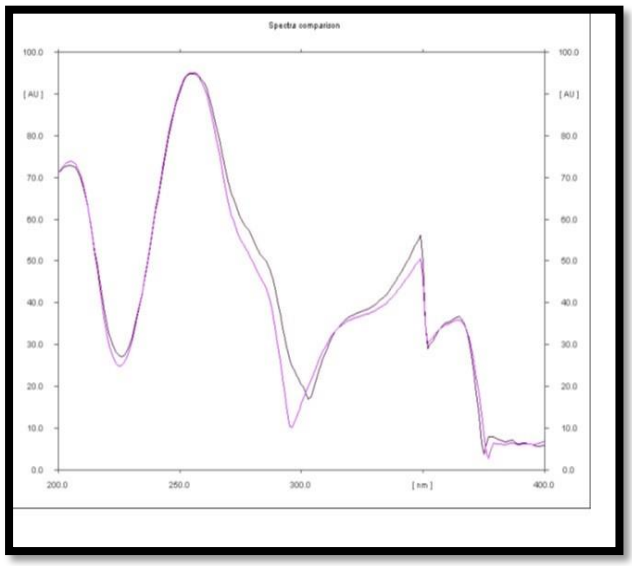

Fig 7. Overlay Spectra of Strychnine

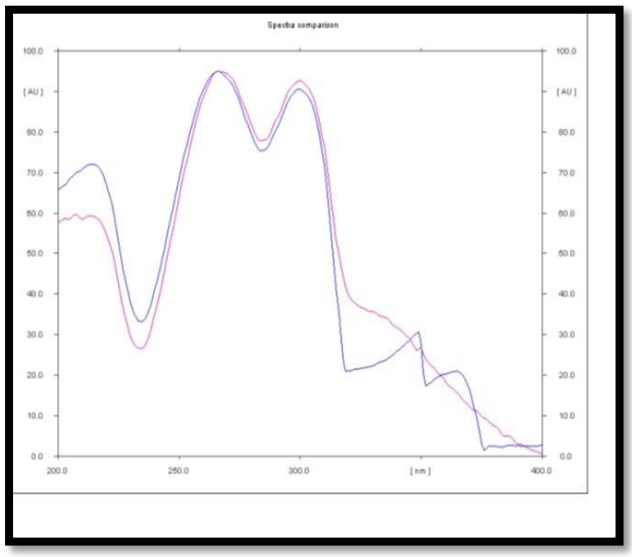

Fig 8. Overlay Spectra of Brucine

Method validation

Specificity

The specificity of the method was established by comparing the *Vishtinduk Vati* extracts against reference standards. By comparing their Rf values and superimposing the peak purity spectra with the spectra of the reference standard, the presence of strychnine and brucine bands in the *Vishtinduk Vati* extract was confirmed.

Linearity

The estimation of the linear relationship between different marker concentrations and peak area response is called linearity. For strychnine and brucine, a linear relationship was observed over the concentration range of 400-900ng/band. The concentration of each marker was found to be directly correlated with the peak area (R2 = 0.999). Table 2

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Table 2:- Linear Regression, LOD & LOQ

Parameters/ Name of Markers	Rf	Linearity Range	Equation	R^2	LOD	LOQ
Strychnine	0.36 ±0.022	400-900 ng/band	y = 165.1x - 41621	0.998	92.76037	281.092
Brucine	0.30 ±0.015	400-900ng/band	y = 103.8x - 20118	0.997	117.78	356.909

Limit of detection (LOD) and Limit of quantification (LOQ)

The standard deviation approach was used to evaluate the limits of detection (LOD) and quantification (LOQ) for each marker. The limits of detection (LOD) and quantification (LOQ) for strychnine and brucine are shown in the table 2.

Precision

The precision of the developed HPTLC method was evaluated through the assessment of intra-day and inter-day precision. The repeatability (intra-day) and intermediate precision (inter-day) were determined by replicate analyses of the marker peak areas (n=6). As summarized in Table 3, the percentage relative standard deviation (% RSD) for all analytes—strychnine and brucine remained consistently below 2.0%, confirming the high degree of method precision.

Table 3:- Precision

Name of Marker	Intra-day				Inter-day		
	Conc. (ng/band)	Mean (n=6)	SD	%RSD	Mean (n=6)	SD	%RSD
Strychnine	600	60378.23	328.97	0.54	62314.98	491.21	0.78
Brucine	600	45367.19	281.98	0.72	45342.53	300.13	0.66

Robustness

The method was found to be robust with respect to changes in mobile phase composition and saturation time (±2 min). The results of the robustness test and % RSD are shown in Table 4.

Table 4:-. Robustness

Sr. No.	Mobile phase composition	Mean Peak ± SD		Duration of saturation time	Mean Peak ± SD	
		Strychnine	Brucine		Strychnine	Brucine
1	2.8:4.2:0.4:1.6	60718.42±452.45	45735.91±311.91	15	60645.31±335.45	45051.54±119.24
2	3:4:0.4:1.6	60378.23±328.97	45367.19±281.98	20	60378.23±328.97	45367.19±281.19
3	3.2:3.8:0.4:1.6	60737.24±421.57	45036.59±312.68	25	60023.24±421.57	45001.15±145.43
	%RSD	0.595	0.770	%RSD	0.517	0.439

Accuracy

Method accuracy was validated via the standard addition method, wherein the formulation extract was spiked with known concentrations of the target markers. The resulting analytical recoveries, presented in Table 5, demonstrate the absence of matrix interference and the high sensitivity of the proposed HPTLC method.

Table. 5:- Accuracy

Marker Name	Spiked Conc. (ng/band)	Measured conc. (ng/band) Mean ±SD	% RSD	% Recovery
Strychnine	400	397.97±0.11	0.14	99.89
	500	499.66±0.20	0.20	99.62
	600	598.73±0.13	0.10	99.66

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Brucine	400	398.75±0.07	0.08	99.72
	500	499.12±0.09	0.09	99.02
	600	597.39±0.09	0.07	99.48

7. Quantification

The HPTLC method was used to estimate the concentrations of strychnine and brucine in *Vishtinduk Vati* extracts. Commercially available *Vishtinduk Vati* was analyzed by densitometry scanning, and linear regression was used to calculate the concentrations of strychnine and brucine. The results are shown in Table 6.

Table. 6 :- Quantification of markers

	Quantification (g/100gm)	
Parameters/ Markers	**Extract of Vishtinduk Vati**	**Crude *Strychnos Nux-vomica* seeds** (17)
Strychnine	0.026± 0.0095	1.25-1.5
Brucine	0.0702±0.090	1.7

5. Discussion

The study evaluated the physical parameters of marketed *Vishtinduk Vati*, revealing low moisture content and a significant amount of inorganic constituents. The total ash value was found to be 19.36%, indicating the presence of inorganic materials in the product. Acid-insoluble ash values were found to be 4.51%, highlighting the presence of inorganic materials insoluble in acid. The water-soluble extractive value was found to be 32%, indicating a significant amount of water-soluble substances in the product. The alcohol-soluble extractive value was found to be 13%, indicating the presence of active constituents soluble in alcohol.

The UV-Vis spectrophotometric study revealed three absorbance peaks in the UV-Vis spectrum, while the solid-state IR (KBr) spectra showed characteristics stretching N-H group, C-H (Ar) group and C=O group. HPTLC examination on silica gel coated plates showed strychnine and brucine in *Vishtinduk Vati* extract as clearly separated spots.

Strychnine and brucine in *Vishtinduk Vati* extracts were analyzed accurately, reliably, and precisely using the HPTLC method. This technique was used to determine the amounts of strychnine and brucine and in the extracts. Compared to raw *Strychnos nux-vomica* seeds, the *Vishtinduk Vati* extract exhibited lower levels of these alkaloids.

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