Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (4), 2022; 1023-1025

Evaluation of seasonal variation in *Veerya* of *visha dravya Kuchala*

Research Article

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Abstract

Introduction - Medicinal plants, as well as poisonous plants, have different alkaloid compositions in every season. According to the traditional texts of *Ayurveda, Visha Dravya* have greater potency in *Varsha Ritu*, which reduces due to *Agasti* rising in *Sharad Ritu*. Objective - To evaluate the potency of *Visha* in *Varsha* and *Sharad Ritu* through estimation of the concentration of Strychnine in *Strychnos nux- vomica L*. Materials and Methods – Quantitative analysis of Strychnine in *Varsha* and *Sharad Ritu* by HPTLC. Results – The concentration of Strychnine was observed higher in *Varsha Ritu* than in *Sharad Ritu.* Conclusion – The study confirms that the potency of *Visha* is greater in *Varsha Ritu* than in *Sharad Ritu*.

Key Words: HPTLC*, Kuchala,* Strychnine*, Visha veerya.*

Introduction

Veerya means potency or active property of any substance. According to *Ayurveda*, variations are observed in medicinal as well as *Visha Dravya*. *Veerya* of plants and the poisons varies with the season. Knowledge of this variation is necessary for pharmaceutical applications. *Acharya Sushruta* has mentioned that the medicinal plants have *Manda Veerya* (Low potency) in *Varsha Ritu* (1). Unlike the medicinal plants, *Visha Dravya* has increased *Veerya* in the *Varsha Ritu*. The origin of *Visha* has been denoted from *Ambu* (Water), leading to increased potency in *Varsha.* The potency is reduced in the *Sharad Ritu* after the rising of *Agasti* star (2) (3). Thus the *Veerya* of *Visha* gets affected by seasons. This gives the indication of seasonal variation in the potency of *Visha*.

This study was undertaken to study this variation in the potency of *Visha* in two different seasons. *Kuchala* is enlisted under the *Upavisha* by *Bhavprakash* and various *Rasa Grantha*. It was selected for the study. Strychnine is the main alkaloid toxic principle present in this *Dravya*.

So, Strychnine was selected to be studied with respect to its quantity in *Varsha Ritu* and *Sharad Ritu* by HPTLC method. The study aims at the validation of basic principles by laboratory analytical study.

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Materials and Methods

Crude Plant Material

Kuchala seeds were collected from Ratnagiri, Maharashtra in *Varsha Ritu* (in the month of August) and again in *Sharad Ritu* (in the month of September) and were authenticated at the Botanical Survey of India , Pune before conducting the experiment.

Standard, Solvents and Additives

Standard sample of Strychnine was procured from Sigma-Aldrich, India. Chloroform, n-hexane, and Methanol were of analytical grade, obtained from Merck, India. Formic acid (85%) was purchased from Loba Chemie, India.

Place of work

Public Testing Laboratory, Pune.

Standard and Sample Preparation

Preparation of standard solution

The stock solution of Strychnine standard was prepared by dissolving 10 mg in 10 mL of methanol (1000 µg/mL).

Preparation of sample solution

Weighed *Strychnos nux-vomica* crushed seed powder was added to methanol (1:10) & sonicated for 45 min for 3 cycles. Then this methanolic mixture was further evaporated using a Rotary evaporator. The residue was further extracted with chloroform (10ml) to precipitate the alkaloids to ensure complete extraction. The chloroform extract was evaporated to dryness on air dry. The residue obtained was reconstituted in chloroform and used for quantification.

HPTLC Instrumentation

An HPTLC system (Camag, Switzerland) equipped with a Linomat V sample applicator fitted with a 100 µL syringe (Hamilton, Switzerland), and a TLC Scanner III operated on WinCats 1.4.4 software (Camag, Switzerland) was used for the analysis. The analysis was performed on precoated silica gel 60 F_{254} aluminium-coated TLC plates (20 cm × 10 cm).

Chromatographic Conditions

Mobile phase

Chloroform: n-Hexane: Methanol: Formic acid (6:2.5:1.5:0.4, v/v/v/v).

Pre-washing and activation of plates

The plates were prewashed with methanol and activated at 60°C for 5 min prior to chromatography. Pre-washing is done to remove impurity and moisture adsorbed on the plates.

Sample Application

A constant application rate of 150 nL/s was employed with a band width of 6.0 mm leaving a gap of 8mm from bottom and 15mm from either sides. The distance between the two bands was kept as 10mm. Six different volumes (0.4, 0.8, 1.2, 1.6, 2.0, 2.4 µL) of strychnine standard solution were applied on a 20 × 10 cm TLC plate for the construction of the calibration curve of strychnine.

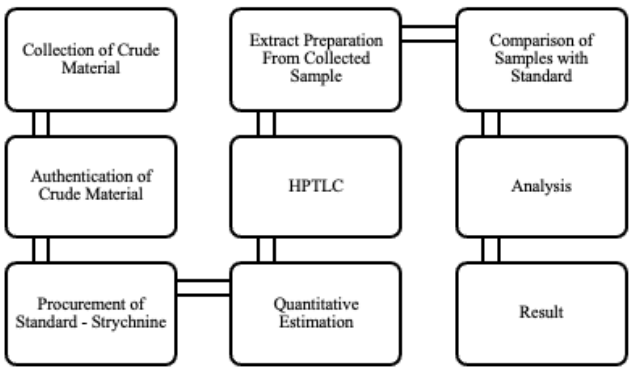
Chromatography development

A 20x10 twin trough chamber was used for development. But before development, the chamber was saturated for 20 mins using Whatmann grade no 1 filter paper. Once the plate was run up to 7 cm, it was removed from the development chamber, dried on the steam of cold air, and then kept the plate on the scanner.

Detection of spots, scanning & documentation

The developed plates were scanned within 10 min using densitometric scanner III in the absorbance mode at 254 for strychnine. The source of radiation was a deuterium lamp emitting continuous radiation between 200–400 nm. The slit dimension was selected as 5mm x 0.45mm and the scanning speed was set at 20mm/s. The data obtained was then subsequently integrated and analyzed using the WinCats software.

Methodology



Observations and Results

Linearity

A 6-point calibration curve was constructed by plotting peak area against different concentrations. Linearity was evaluated by applying each concentration (400–2400 ng/spot) for strychnine in triplicates per sample and 6 such samples were evaluated (n=3×6).

The equation obtained from linearity data has been used for quantification of the Strychnine present in the sample extract.

Y= mx+C,

The equation obtained is, Y = 4.997x + 2540 (r^2=0.980)

Where, Y- area under curve

x - Concentration of strychnine

Table no 1. Enlists the different concentrations of Strychnine Standard (ng/spot) along with its respective Area (AU)

Concentration (ng/spot)	Area (AU)
413.2	3865.3
826.4	6978.2
1239.6	9562.6
1652.8	10855.2
2066.0	12740.1
2479.2	14603.4

Figure 1. Calibration curve of Strychnine standard with varying concentration plotted on the X-axis and its respective area under the curve plotted on Y-axis

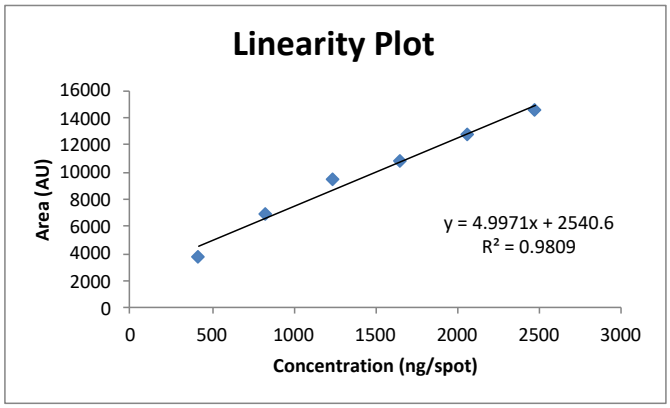


Figure 2 : HPTLC densitogram of

A- *Strychnine* Standard | **B-*Strychnos Nux-Vomica* seed extract in *Varsha Ritu*** | ***C. Strychnos Nux-Vomica* seed extract in *Sharad ritu***

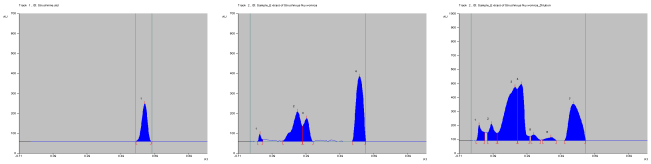


Table no 2 . Enlisting the R_f values, Area and % Strychnine content present in standard *Strychnine* sample, *Strychnos Nux-Vomica* seed extract in *Varsha Ritu and Sharad Ritu*

Sr. No.	Sample	R_f Value	Area (AU)	% of Strychnine present (By HPTLC)
1	Strychnine_Standard	0.63	4964.6	-
2	*Strychnos Nux-Vomica* seed extract in *Varsha Ritu*	0.61	10949.2	3.67
3	*Strychnos Nux-Vomica* seed extract in *Sharad Ritu*	0.56	14108.8	1.33

Discussion

Ayurveda classical texts mention the variation in the *Veerya* of Visha and the *Veerya* increases in *Varsha Ritu* and decrease in *Sharad ritu*. This study was conducted in order to study the variation of the potency of *visha dravya* in *Varsha Ritu* and *Sharad Ritu*. *Kuchala* being one of the *Upavisha* was selected for the study. Strychnine is one of its active principles. As the action of *Kuchala* is mainly due to this alkaloid, this was selected for the study. Quantitative analysis of Strychnine was done by using the HPTLC method.

It was observed that the quantity of active principle Strychnine was more in *Varsha Ritu* than in the *Sharad Ritu.* This proves the objective of this study.

Further scope of the study

In this study, the potency of poison was studied in *Varsha* and *Sharad Ritu*. This study can further be conducted throughout the year in six different seasons. Other poisons should be studied for the seasonal variations. On the basis of this, the formulations in which *Kuchala Visha* is used as an ingredient should be prepared in the season when the toxic content of *Strychnine* is low. It can be applied as an SOP where *Kuchala* is used as one of the ingredients.

Conclusion

After the analysis of Strychnine with the help of HPTLC, the quantity of Strychnine was observed more in *Varsha Ritu* and the amount got reduced in the *Sharad Ritu*. The present study confirms that the potency of poison is more in the *Varsha Ritu* and it gets decreased in the *Sharad Ritu*. The limitations of this study are that the study was conducted in two seasons. This study needs to be extended throughout the year.

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