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Evaluation of Ethanol Extracts from *Strychnos nux-vomica* L. using High-Performance Thin Layer Chromatography (HPTLC)

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Abstract: *Strychnos nux-vomica* L., commonly known as the Nux-vomica tree, is renowned for its therapeutic properties within the Indian traditional medicinal system. This study aims to analyze the phytochemical profile of ethanol extract of *Strychnos nux-vomica* through High-Performance Thin Layer Chromatography (HPTLC). The HPTLC densitometric analysis was conducted using a CAMAG HPTLC system, with chromatograms scanned at wavelengths of 254 nm and 366 nm, revealing multiple peaks indicative of various bioactive compounds. The phytochemical profiling was documented in tables detailing the total number of peaks, peak heights, peak areas, percent areas, and Rf values. The results demonstrate that the ethanol extract from the stem of *Strychnos nux-vomica* is rich in diverse phytochemicals, which are likely responsible for its therapeutic efficacy. This finding substantiates the traditional usage of this plant in India and enhances our understanding of its pharmacological potential and safety considerations associated with its medicinal applications. The study contributes significantly to the body of knowledge regarding *Strychnos nux-vomica*, emphasizing its value in herbal medicine and laying the groundwork for further research into its bioactive constituents and their mechanisms of action.

Keywords: *Strychnos nux-vomica*, HPTLC, Ethanol extract, Phytochemical analysis, Therapeutic properties

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Introduction

India has one of the oldest, richest, and most diverse cultural traditions associated with the use of medicinal plants (Joshi *et al*., 2018). Many of these plants have been traditionally used for thousands of years and form an integral part of Ayurveda, the Indian traditional healthcare system. These herbal preparations are recognized for their diverse therapeutic activities. While some medicinal plants have been extensively studied, others still require detailed scientific exploration (Kumar and Sharma, 2020). The phytochemical evaluation of plants with a well-documented

history in folklore medicine has often led to the isolation of bioactive compounds with significant pharmacological properties (Patel and Singh, 2017). High-Performance Thin Layer Chromatography (HPTLC) is a crucial tool for developing chromatographic fingerprints, which help in the identification and quantification of major active constituents in medicinal plants. Compared to conventional Thin Layer Chromatography (TLC), HPTLC offers superior separation, resolution, and reproducibility. Additionally, the integration of digital scanning profiling allows for precise qualitative and quantitative measurements through densitometry (Verma *et al*., 2019).

Strychnos nux-vomica L., commonly known as the strychnine tree, is a notable medicinal plant indigenous to Southeast Asia and Australia. The plant's seeds and bark have been used in traditional medicine for various ailments, including neurological disorders, muscle spasms, and as a stimulant. The primary active constituents of *Strychnos nux-vomica* are alkaloids, particularly strychnine and brucine, which are responsible for both its therapeutic effects and toxicity. Strychnine is a potent neurotoxin that can cause severe convulsions and death in high doses, while brucine has milder effects and is often studied for its potential medicinal properties.

The extraction of bioactive compounds from medicinal plants is essential for their standardization and quality control. High-Performance Thin Layer Chromatography (HPTLC) is a powerful analytical technique that allows for the separation, identification, and quantification of phytochemicals in complex mixtures. HPTLC offers several advantages, including high resolution, speed, and the ability to analyze multiple samples simultaneously.

This study aims to evaluate the ethanol extracts of *Strychnos nux-vomica* using HPTLC to determine the presence and concentration of its major alkaloids, thereby contributing to the understanding of its pharmacological potential and safety profile.

Materials and Methods

Plant Collection

Plant materials were collected from Pachaimalai Hills, Tiruchirappalli District, Tamil Nadu, India. The species were identified and authenticated at the Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Preparation of Extract

The collected plant material was washed thoroughly and separated into leaf, bud, and stem. These parts were shade-dried at room temperature to retain their phytochemical properties. The dried plant materials were then powdered using a mechanical grinder. The powdered material was extracted using ethanol (Patel and Mehta, 2019). The obtained plant extract was used for phytochemical analysis.

Phytochemical Studies

Plants produce a diverse range of bioactive compounds such as alkaloids, flavonoids, steroids, saponins, phenols, proteins, cardiac glycosides, and carbohydrates, many of which possess pharmaceutical properties. The plant extract was subjected to preliminary phytochemical screening (Sharma *et al*., 2017; Verma and Gupta, 2018).

Test for Alkaloids

- *Mayer's Test*: Few drops of Mayer's reagent were added to the test solution. The formation of a yellowish or white precipitate indicated the presence of alkaloids**.**
- *Wagner's Test*: Few drops of Wagner's reagent were added along the sides of the test tube containing the extract. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for Flavonoids

- *Alkaline Reagent Test*: The test solution was mixed with sodium hydroxide (NaOH). A deep yellow color appeared, which became colorless upon the addition of dilute HCl, indicated the presence of flavonoids.

- *Shinoda's Test*: Dilute HCl (10 drops) and a small piece of magnesium were added to 1 ml of extract. The formation of a deep pink color indicated the presence of flavonoids.

Test for Steroids

- *Libermann-Burchard's Test*: 2 ml of acetic anhydride was added to the test solution, followed by 1-2 drops of concentrated H_2SO_4 along the sides of the test tube. The appearance of color changes indicated the presence of steroids.

Test for Saponins

- A drop of sodium carbonate (Na_2CO_3) solution was added to the test solution and vigorously shaken. The formation of foam indicated the presence of saponins.

Test for Phenols

- *Ferric Chloride ($FeCl_3$) Test:* 1 ml of water was added to the test solution, followed by 1-2 drops of $FeCl_3$. The appearance of a blue or green color indicated the presence of phenols.

Test for Proteins

- *Biuret Test*: Few drops of 3% copper sulfate ($CuSO_4$) and 10% sodium hydroxide (NaOH) were added to 1 ml of extract. The formation of a red or violet color confirmed the presence of proteins.

Test for Cardiac Glycosides

- *Keller-Killiani Test*: Glacial acetic acid and 2-3 drops of ferric chloride were mixed with 2 ml of extract. Later, 1 ml of concentrated H_2SO_4 was added along the walls of the test tube. A reddish-brown color confirmed the presence of cardiac glycosides.

Test for Carbohydrates

- The test solution was mixed with 5 ml of distilled water and filtered. The absence of color change indicated the absence of carbohydrates.

High-Performance Thin Layer Chromatography (HPTLC)

Chromatographic Fingerprint Standardization

The standardization of crude drugs is essential to establish their correct identity and authenticity. HPTLC analysis provides a chromatographic fingerprint profile, which is crucial for the quality control of herbal extracts.

Sample Preparation and Application

- A 1 mg/ml concentration of the extract was prepared using chromatographic-grade ethanol and filtered through Whatman filter paper No. 1.
- The extract was sonicated and centrifuged before application.
- Serial dilutions of the extract (5, 10, 15, 20 µl) were applied to TLC aluminum sheets (silica gel 60 F 254, Merck) using a Linomat 5 sample applicator at a speed of 100 nl/sec.

Developing Solvent System

Several solvent systems were tested to achieve optimal separation and resolution. The best results were obtained with Toluene: Ethyl acetate (9:1 v/v)

Development of Chromatogram

- The chromatograms were developed in a twin-trough glass chamber saturated with the solvent system (Toluene: Ethyl acetate 9:1 v/v) for 20 min, allowing a migration distance of 70 mm.

Scanning and Detection of Spots

- The air-dried plates were observed under ultraviolet light (254 nm and 366 nm).
- Visible light (400-600 nm) was used for further detection.
- Scanning was performed using a CAMAG HPTLC Densitometer (Scanner 4) in absorbance mode at 254 nm and 366 nm, using deuterium and tungsten lamps with a slit dimension of 6.0 × 0.45 µm.
- The Rf values and colors of the resolved bands were recorded.

Results and Discussion

The phytochemical test on ethanol extract of *Strychnos nux-vomica* revealed the presence of various bioactive compounds, including alkaloids, flavonoids, steroids, saponins, phenols, proteins, cardiac glycosides, and carbohydrates.

Chromotographic Analysis

The HPLC analysis was conducted at a wavelength of 366 nm, and the results are summarized in the chromatograms presented (Figs. 1-7). The retention factors (Rf) for the compounds analyzed were calculated and are illustrated in the graphs.

Each compound exhibited distinct retention times, indicating varying degrees of interaction with the stationary phase. The peak areas were measured to quantify the concentration of each compound in the sample.

The evaluation of ethanol extracts from *Strychnos nux-vomica* L. using High-Performance Thin Layer Chromatography (HPTLC) provides valuable insights into the phytochemical profile of this medicinal plant. The results, which include the retention factor (Rf) values for five distinct peaks at a wavelength of 366 nm, highlight the complex composition of the extract (Table 1).

Analysis of Peak Data

These values indicate varying degrees of polarity and interaction with the stationary phase, which is critical for understanding the chemical constituents present in the extract.

Peak Characteristics

Peak 1 (*Rf = 0.168*) *and Peak 5* (*Rf = 0.125*): The low Rf values suggest that these compounds are more polar and have a stronger affinity for the stationary phase. This could indicate the presence of alkaloids or other polar compounds known to be characteristic of Strychnosnux-vomica.

Peak 2 (*Rf = 0.443*) *and Peak 3* (*Rf = 0.590*): These intermediate Rf values suggest a moderate polarity, possibly indicating the presence of glycosides or flavonoids, which are often found in plant extracts and can exhibit various biological activities.

Peak 4 (Rf = *0.778*): The relatively high Rf value indicates a less polar compound, which may correspond to non-polar constituents such as fatty acids or terpenoids.

The diversity in Rf values suggests that *Strychnos nux-vomica* contains a range of bioactive compounds that could contribute to its pharmacological properties. Previous studies have indicated that this plant is rich in alkaloids, particularly strychnine and brucine, which are known for their neurotoxic effects but also possess potential therapeutic benefits when used in controlled doses.

The retention factor (*Rf*) values obtained from the HPTLC analysis indicate a diverse range of compounds with varying polarities. Low *Rf* values for Peak 1 (0.168) and Peak 5 (0.125) suggest that these compounds are more polar and likely have stronger interactions with the stationary phase. These peaks may correspond to alkaloids, which are characteristic of *Strychnos nux-vomica*. Alkaloids such as strychnine and brucine are known for their significant pharmacological effects, including neurotoxicity and potential therapeutic applications in controlled doses (Kumar and Sharma, 2019).

Peaks 2 (0.443) and 3 (0.590) represent compounds with moderate polarity, potentially indicating the presence of flavonoids or glycosides. These compounds are often associated with various biological activities, including antioxidant and anti-inflammatory effects (Patel *et al*., 2020). The higher Rf value for Peak 4 (0.778) suggests a less polar compound, which could be indicative of terpenoids or fatty acids. These compounds can contribute to the overall therapeutic profile of the plant by providing additional health benefits (Verma and Gupta, 2018).

The presence of diverse compounds highlights the complex phytochemical profile of *Strychnos nux-vomica* and underscores its potential as a source of bioactive substances. Previous research

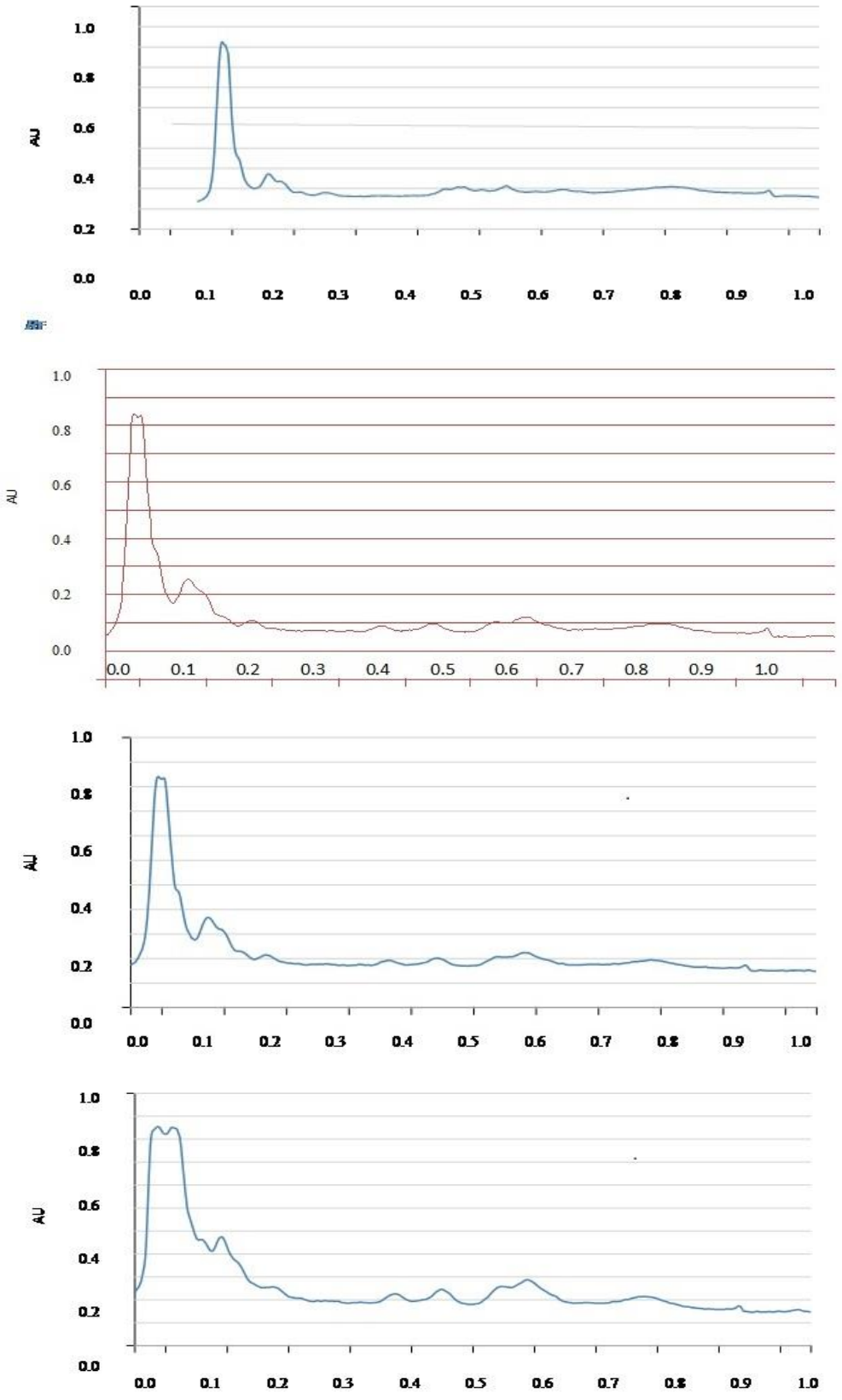

Fig. 1: HPTLC Chromatograms Wave length at 254 nm.

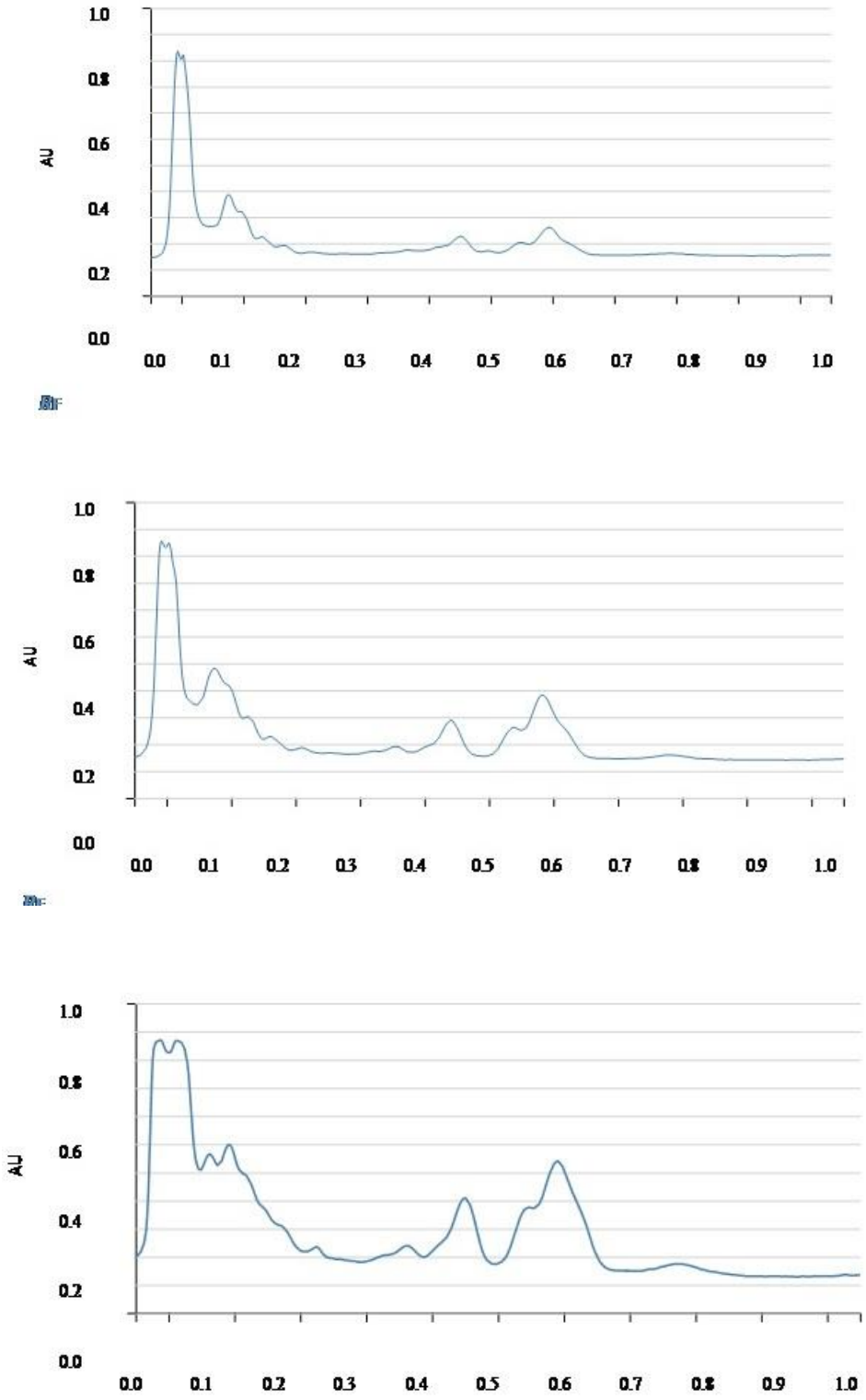

Fig. 2: HPTLC Chromatograms Wave length at 366 nm.

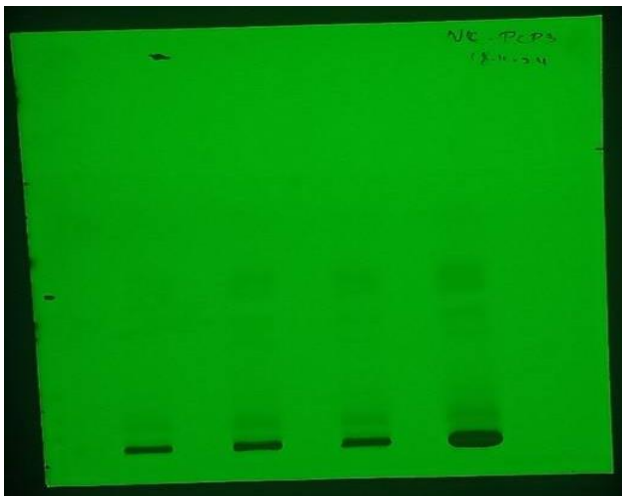

Fig. 3: HPTLC Chromatogram viewed at 254 nm.

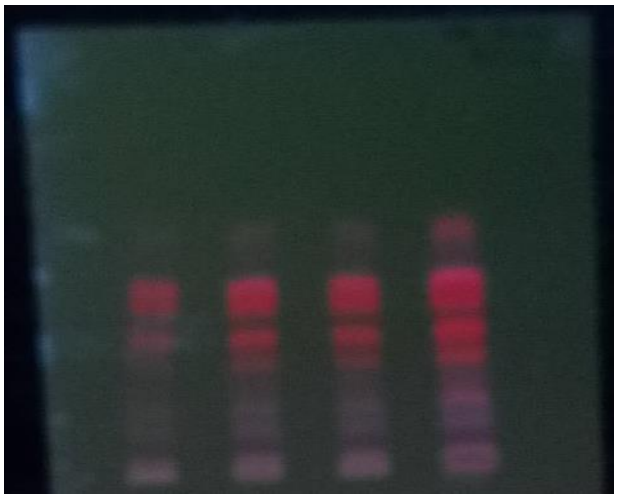

Fig. 4: HPTLC Chromatogram viewed at 366 nm.

Fig. 5: HPTLC Chromatogram viewed at 366 nm.

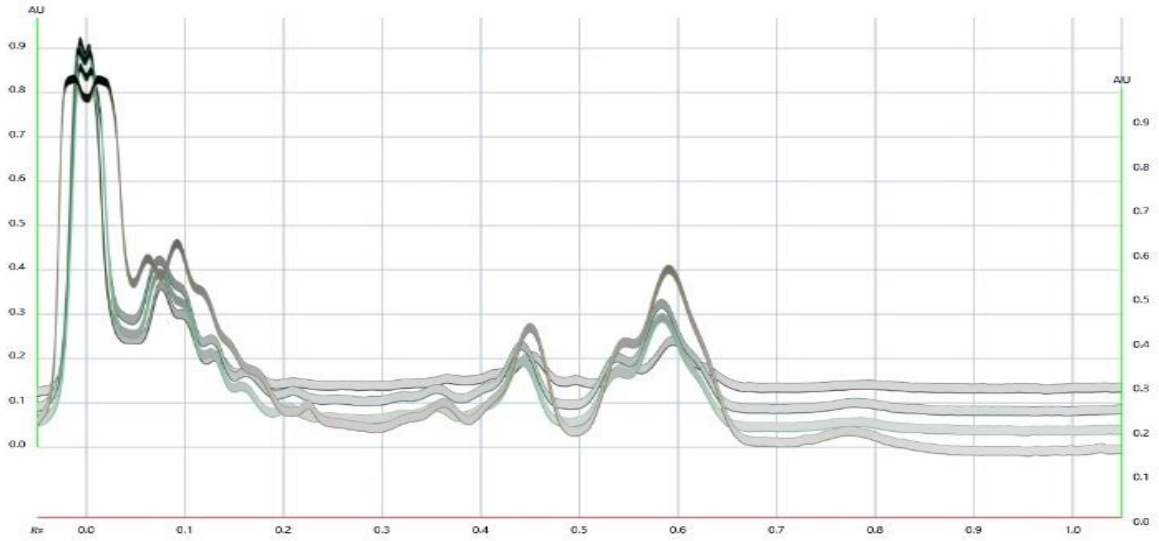

Fig. 6: HPTLC of unknown crude drug ethanolic extract (All tracks at wavelength) 366 nm.

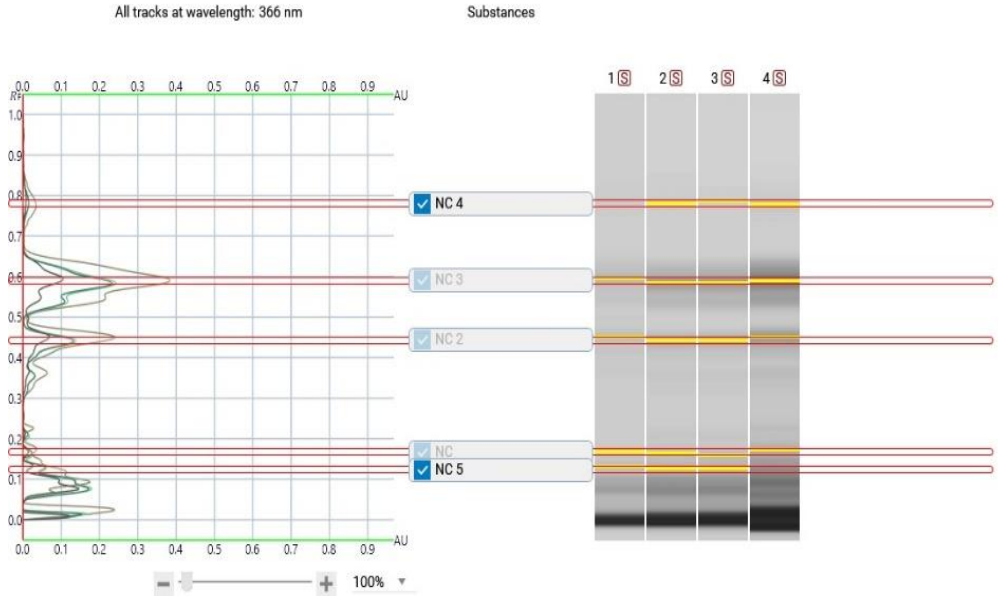

Fig. 7: HPTLC of unknown crude drug extract (All tracks at wavelength) 366 nm.

Table 1: Data showing the R_f values of unknown crude drug ethanolic extract at 366 nm

S. No.	PEAK No.	λ	R_f value of sample
1.	Peak 1	366 nm	0.168
2.	Peak 2		0.443
3.	Peak 3		0.590
4.	Peak 4		0.778
5.	Peak 5		0.125

has established that extracts from this plant have been used in traditional medicine for treating various ailments, including neurological disorders, due to the effects of its alkaloids (Singh and Mehta, 2017).

The findings from this study suggest several avenues for future research:

- Further studies should focus on isolating individual compounds corresponding to each peak for detailed characterization using advanced techniques such as Mass Spectrometry (MS) or Nuclear Magnetic Resonance (NMR) spectroscopy.
- It is crucial to evaluate the pharmacological activities associated with each identified compound through bioassays, particularly focusing on their therapeutic potential and toxicity profiles.
- Establishing standardized extraction protocols can enhance the reproducibility and efficacy of extracts used in pharmaceutical applications.

Conclusion

In conclusion, the HPTLC analysis of ethanol extracts from *Strychnos nux-vomica* has revealed a rich array of phytochemicals with varying polarities, indicating significant potential for therapeutic applications. This study not only contributes to the understanding of the chemical composition of this medicinal plant but also emphasizes the need for further research to explore its full pharmacological potential.

Conflict of Interest

The authors declare no conflicts of interest.

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