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Research Article

Analysis of Strychnine and Brucine in hydro ethanolic extract of Strychnos Nux-Vomica leaves by using HPTLC

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ABSTRACT

Strychnos Nux-Vomica is a remedial plant used in herbal medicine and medicinal plants is having a curative history and are still helping healthiness requirements of a large population in the world. Therapeutic plants are used to cure various diseases, these are the phytochemicals extracted from plants. *Strychnos-nux-vomica* is having Strychnine and Brucine with biological properties. However, qualitative and quantification of the compounds in plant extract is determined by using chromatography techniques. The method HPTLC is used in identification, standardization and quantification of bioactive Phytoconstituent in plant extract and these extracts helpful in medications. *Strychnos Nux-vomica* leaves are extracted with 50% Ethanol in a water and centrifuged at 3000 rpm for 5min for analysis. 3 µl of Sample S and 2 µl of standard solution were loaded in CAMAG LINOMAT 5 instrument and sc anning done at UV 254nm. The Mobile phase is used ratio of Toluene – Ethyl acetate – Diethyl amine (7:2:1) and Dragendorff's reagent is used as spraying for spot development. HPTLC chromatograms shows the Strychnine, Brucine and eight unknown compounds. Strychnine, Brucine Rf values are 0.50, 0.33 and values of the area is 16667.7 and 13826.4.

Keywords: Brucine; HPTLC; Strychnine; Strychnos Nux-Vomica.

INTRODUCTION

Therapeutic plants are ancient and used in numerous ways for the treatment of different illnesses. They will be extracted forms phytochemicals from the plants (Patel *et al*., 2011). Ethno therapeutic study deals with the study of traditional medications. Since ancient times humans using herbal plants, organic materials for treatment of illness, these are collected nature. Several portions of the plants like roots, leaves, bark, exudates are having medicinal properties (Samy and Krishnakone, 2007). Chromatographic techniques used in qualitative and quantitative analysis for herbal medicines to produce good quality products (Gong *et al*., 2005). Since they are natural integrities of the herbal medications and its products, used for authentication and identification of herbal plant (Liang *et al*., 2005). Treatment of wellbeing complaints, phytochemicals are isolated from plants. These phyto medicines used African and Asian populations as medicines for their health care issues (Bozzi *et al*., 2005).

Strychnos-nux-vomica is a medicinal plant, belongs to *loganiacea* family *and* Common are Kanjiram, Kuchla, Kupilu (Harry, 1968).Phytochemicals are mainly collected from the plants and for the colour, flavor and smell of the plants. They are beneficial values for human health (Eleauzu *et al.,* 2005). HPTLC profile is an analytical tool and powerful procedure to find the presence or absence of chemical constituents in plants. In Pharmacognosy, raw materials analysis and final product analysis will be performed by using HPTLC. Development, optimization and Standardization procedures and pharmacognostical studies of medicinal plants for traditional herbal medicines (Dinesh Kumar, 2007).

MATERIALS AND METHODS

Plant Collection & authentication of Plant

Leaves of *Strychnos Nux-vomica* was used for investigation obtained from Nellore district, Andhra Pradesh, India. The plant was authenticated by G.V.S Moorthy, Botanical survey of India, Coimbatore. Authentication number of plant is BSI/SRC/5/23/2013-14/Tech/682.

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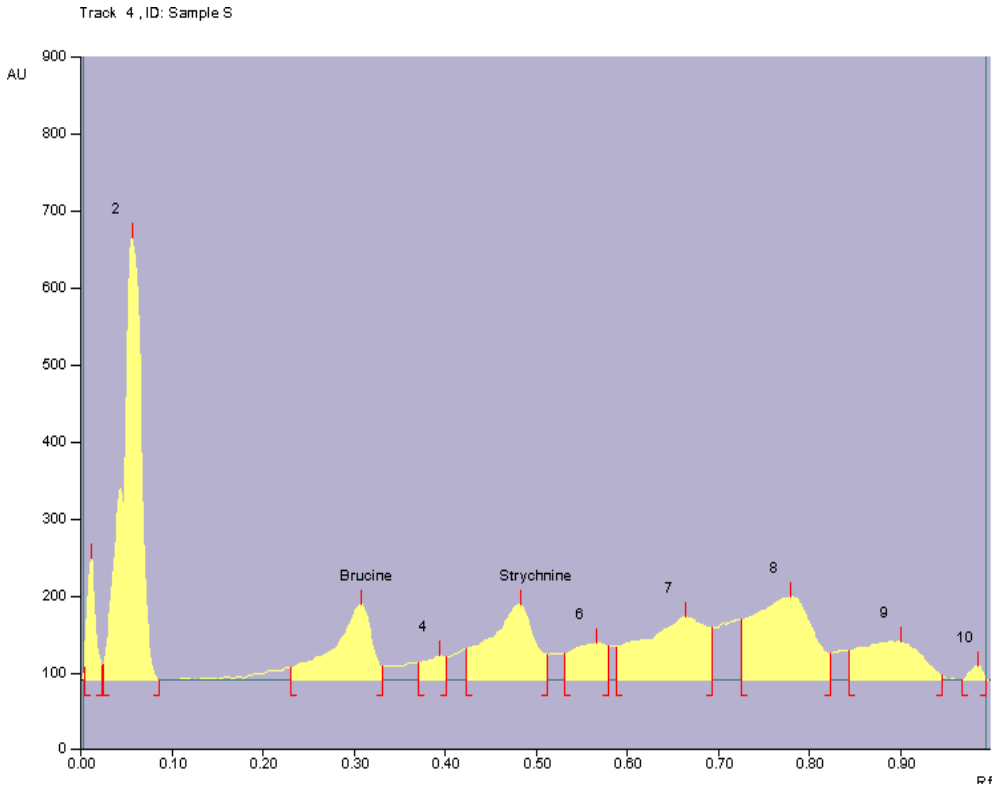

Figure 1: HPTLC chromatograms shows the compounds of Strychnos Nux-vomica.

Table 1: Compounds present in *Strychnos Nux-vomica* leaves extract of the HPTLC analysis

Sample ID	Peak	Rf	Height	Area	Assigned substance
STD 1	1	0.50	440.5	16667.7	Strychnine standard
STD 2	1	0.33	488.2	13826.4	Brucine standard
Sample S	1	0.01	126.9	512.2	Unknown
Sample S	2	0.06	565.6	10344.9	Unknown
Sample S	3	0.31	98.9	3702.8	Brucine
Sample S	4	0.39	31.3	692.5	Unknown
Sample S	5	0.48	98.4	4421.4	Strychnine
Sample S	6	0.57	48.2	1675	Unknown
Sample S	7	0.66	81.5	5351.9	Unknown
Sample S	8	0.78	108	6581.4	Unknown

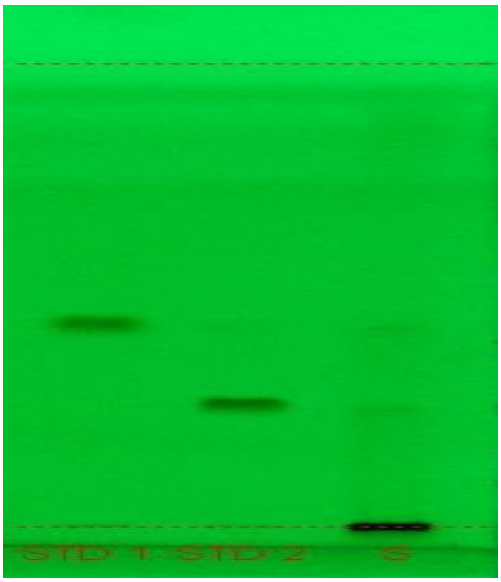

Figure 2: HPTLC chromatograms of *Strychnos Nux-vomica* at 254nm.

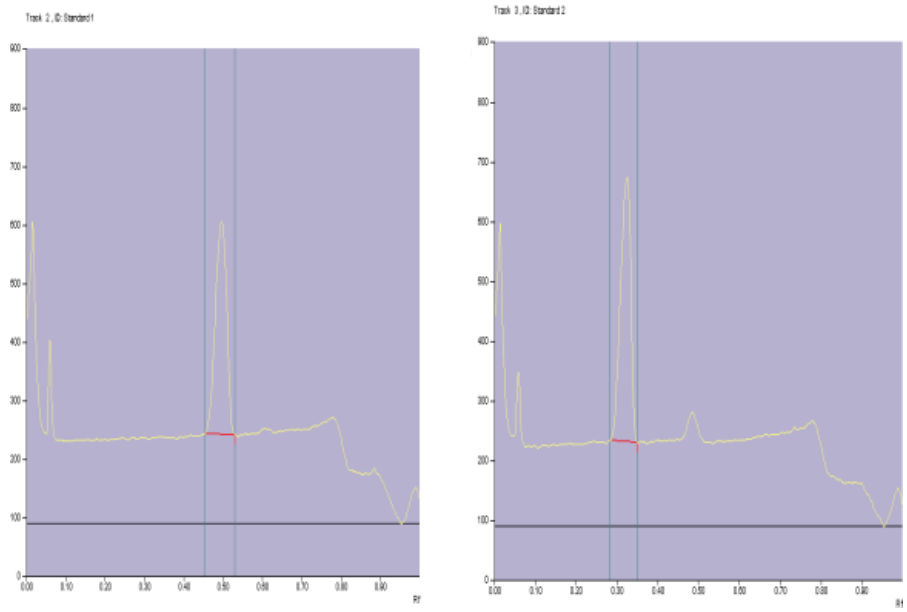

Figure 3: STD 1 and STD 2 – Alkaloid standard Baseline display (Scanned at 254nm)

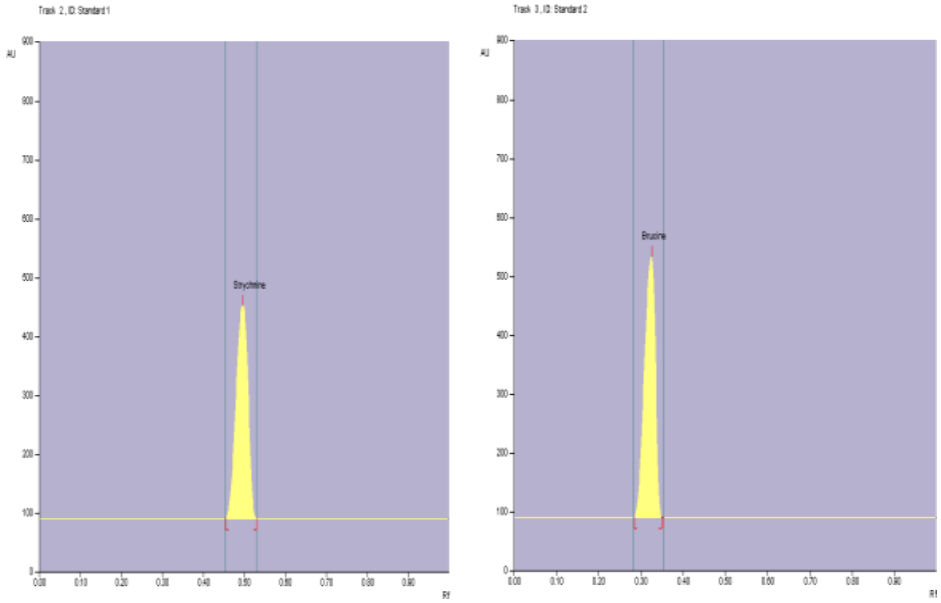

Figure 4: STD 1 and STD 2 – Alkaloid standard Peak densitogram display (Scanned at 254nm)

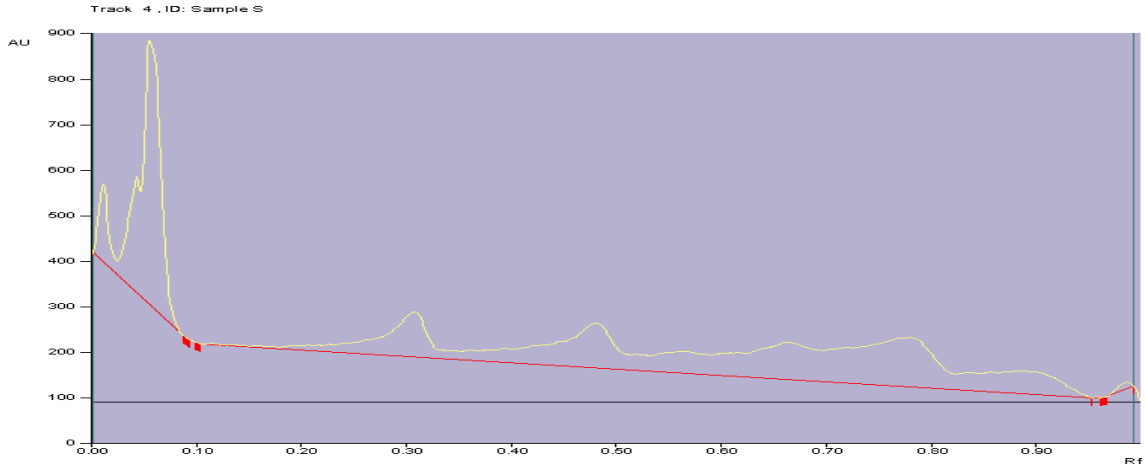

Figure 5: Sample S – Plant sample Baseline display (Scanned at 254nm)

Extraction Procedure

Leaves of Strychnos Nux-vomica is washed with distilled water, shade dried, powered for the solvent extraction process. The crude extract was obtained by extracting 50 grams of dried plant powder in 200ml of 50% Ethanol in a water shaker for 72 hrs. Repeatedly solvent extraction was done with the same solvent till colour less solvent obtained. The hydro ethanolic plant extract was further concentrated by using Rota evaporator at 45-50 °C. After concentration, the residue occurred was dissolved in methanol and analysis is carried out by using HPTLC.

Test solution and standard preparation

The given plant sample centrifuged at 3000rpm for 5min. This solution was used as a test solution for HPTLC analysis. The given standards were prepared-with the concentration of 1mg/1ml in Methanol.

Sample and standard application

3 µl of Sample S and 2 µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using a Hamilton syringe and CAMAG LINOMAT 5 instrument.

Standard and Reagents

Strychnine, Brucine, Toluene, Ethyl acetate, Diethyl amine, Ethanol are procured Sigma Aldrich, Bangalore, India.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Alkaloid) and the plate was developed in the respective mobile phase up to 90mm.

Scanning

Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version.

Mobile phase

The Mobile phase is used ratio of Toluene – Ethyl acetate – Diethyl amine (7:2:1) and Spray reagent is Dragendorff's reagent is used for Spray reagent.

Sample Analysis

Black colored quenching zones observed in UV 254nm both in the standards and in the sample indicates the Presence of Alkaloid (Strychnine and Brucine).

RESULTS AND DISCUSSION

This HPTLC study of Ethanolic extract of *Strychnos Nux Vomica L*. showed 8 peaks at a concentration of 1mg/ml of the sample and 10 peaks including spiked standard (Figure 1 and Table 1). Rf and Area of the sample are mentioned in Table 1. 2µl of Brucine and strychnine standard solution is used for analysis. 3µl of sample extract is used for sample analysis. Figure 2 shows the HPTLC cartogram of *Strychnos Nux-vomica* at 254nm.Figure 3 and 4 shows the Baseline display and densitogram display of Strychnine and brucine standard at 254nm as well sample baseline display is mentioned in Figure 5. HPTLC fingerprinting is a tool for the assessment of botanical materials and this method is simple, rapid, accurate, reproducible, selective and economic, can be used for qualitative and quantitative analysis (Wagner *et al*., 1996).

CONCLUSION

Strychnine and Brucine active compounds in Strychnos Nux-Vomica leaves were identified by using HPTLC with against the Standard. These alkaloids plays crucial role in pharmaceutical and biological industries.

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