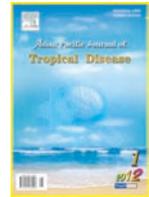




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Document heading

## Phytochemical analysis and standardization of *Strychnos nux-vomica* extract through HPTLC techniques

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## ABSTRACT

**Objective:** The objective is to develop a novel qualitative and quantitative method by which we can determine different phytoconstituents of *Strychnos nux-vomica* L. **Methods:** To profile the phytoconstituents of *Strychnos nux-vomica*, in the present study hydroalcoholic extract of *Strychnos nux-vomica* was subjected to preliminary phytochemical analysis, antimicrobial activities against certain pathogenic microorganisms, solubility test, loss on drying and pH value. Extract was also subjected to the quantitative analysis including total phenol, flavonoid and heavy metal analysis. Quantitative analysis was performed through HPTLC methods using strychnine and brucine as a standard marker. **Results:** Phytochemical analysis revealed the presence of alkaloid, carbohydrate, tannin, steroid, triterpenoid and glycoside in the extract. Total flavonoid and phenol content of *Strychnos nux-vomica* L extract was found to be 0.40 % and 0.43%. Result showed that the level of heavy metal (lead, arsenic, mercury and cadmium) comply the standard level. Total bacterial count, yeast and moulds contents were found to be under the limit whereas *E. coli* and *salmonella* was found to be absent in the extract. Content of strychnine and brucine were found to be 4.75% and 3.91%. **Conclusions:** These studies provide valuable information for correct identification and selection of the drug from various adulterations. In future this study will be helpful for the quantitative analysis as well as standardization of the *Strychnos nux-vomica* L.

### 1. Introduction

Phytochemicals are compounds that occur naturally in plants. They are responsible for different color, flavor and smell of plants. They form part of a plant's natural defense mechanism against diseases. Their therapeutic values to human health have been reported in the different system of medicine [1]. Natural products such as pure phytoconstituents and plant extracts offer limitless opportunities for new drug development due to the unmatched availability of chemical diversity. Plants play an important role in the medicinal preparations, both preventive and curative. In the world, China and India are the leading countries in using medicinal plants and their

traditions of plant remedies date back to at least 7000 years. According to WHO 80% of the World's population relies on traditional medicine to meet their daily health requirement. Today the vast traditional knowledge of medicinal plants is playing an important role in the development of new drugs. Some of the important drugs which were developed from plants source are aspirin from filipendula ulmar, morphine from papaver somniferum and ephedrin from ephedra [2]. A large number of phyto drugs prescribed worldwide are derived directly or indirectly from natural sources. A number of plant based drug is included in the WHO's essential medicine list [3].

*Strychnos nux-vomica* is an evergreen tree native to South East Asia and India belonging to family Loganiaceae. It is a medium size tree found mostly in open habitats. Strychnine (C<sub>21</sub>H<sub>22</sub>O<sub>N</sub><sub>2</sub>) and brucine (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>) are the two main poisonous alkaloids found union with other phytoconstituents such as igasuric acid and loganin. It is

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cultivated commercially in the different part of world such as United States, European Union, Fujian, Guangdong, Guangxi, Hainan, Taiwan, and throughout tropical Asia. According to the U.S.P., content of alkaloid should not be less than 2.5 % in *Strychnos nux-vomica* [4]. At low dose level it is used as stimulant, laxative and for the treatment of other stomach ailments but in higher doses it is toxic. Pharmacologically *Strychnos nux-vomica* showed anticancer, antimicrobial, antiinflammatory, antioxidant, and antifeederent activity. Their specific effects on gastrointestinal problem, nervous system, blood glucose level, bones cells and cardiovascular systems have been also investigated [5]. Development of various novel analytical techniques for the analysis of medicinally significant phytoconstituents has led to the resurgence in this area of research. *Strychnos nux-vomica* has been chosen for the present study. The overall objective has been to develop novel qualitative and quantitative techniques, which can pave the way for rapid and selective determination of different constituents of *Strychnos nux-vomica*.

## 2. Material and methods

### 2.1. Plant extract and Chemicals

Crude plant extract was procured from Garlico Herbal Concentrate (M.P.), India. HPTLC precoated plates Silica Gel Merck 60F254 was used as a stationary phase, strychnine and brucine were used as a marker compound. All the chemicals and reagents used in the present analysis of analytical grade.

### 2.2. Development of analytical methods

A phytochemical screening was conducted on the *Strychnos nux-vomica* seed extracts using standard qualitative methods to confirm the presence of phytoconstituent. Preliminary phytochemical analysis of hydro alcoholic extract was done [6, 7]. The presence of different active constituents was also analysed through TLC analysis [8]. Solubility, loss on drying, heavy metal and microbiological analysis were performed according to the IP, 1996 and WHO guidelines [9, 10]. Total phenol and flavonoid content

were also determined according to the standard methods [11, 12]. Aluminum chloride colorimetric method was used for the total flavonoid determination. Quercetin was used as a standard at concentrations of 12.5 to 100  $\mu$ g/ml in methanol. Different combination of solvent system has been used for the optimization of suitable solvent system for quantitative analysis through HPTLC methods. The quantification of strychnine and brucine in *Strychnos nux-vomica* was determined by High Performance Thin Layer Chromatography (HPTLC) manufactured by CAMAG. The quantity (%w/w) of strychnine and brucine were calculated according to the standard method. Different concentration of standard solution of marker compound (strychnine and brucine) was applied on HPTLC plates along with methanolic extract of *Strychnos nux-vomica*. The HPTLC plates were developed in a suitable solvent system and dried in air and scanned densitometrically at 254 nm. The method was validated in terms of precision and accuracy. The HPTLC chromatography condition for the analysis is as follows

## 3. Results

Crude herbal extract (hydro alcoholic) was taken for this purpose, colour of extract was brown and organoleptic test was found to be bitter. Preliminary Phytochemical analysis showed that alkaloid, carbohydrate, tannin, steroid, triterpenoid, glycoside, and were present and saponin, flavonoid amino acid, protein was absent. TLC analysis showed four spots R<sub>f</sub> (0.36, 0.49, 0.55, 0.82) in n-butanol: acetic acid: H<sub>2</sub>O (4:1:5), solvent system and alkaloid was found to be present. pH of the 1% solution was found to be 4.98, loss on drying was 4.35%, where as solubility in water was 73%, The total flavonoid and phenol content of *Strychnos nux-vomica* L extract was found to be 0.40 % and 0.43%. Further heavy metal analysis was performed and result showed that the level of lead, arsenic, mercury and cadmium complies the standard level i.e. lead <10 PPM, arsenic and mercury <1 PPM and cadmium <0.1 PPM. Microbiological assay was also performed in the current task and the result showed that total bacterial count, yeast and moulds contents were found to be under the limit whereas *E. coli* and *salmonella* was found to be absent in the extract.

Fingerprinting analysis of sample was done through

1	Analysis:	Estimation of strychnine and brucine in <i>Strychnos nux-vomica</i> extract.
2	Plate material:	HPTLC Precoated plates Silica Gel Merck 60F254
3	Solvent system:	Toluene: Ethyl acetate: Diethyl amine (70:20:10)
4	Syringe:	100 $\mu$ L Hamilton (Bonadzu, Switzerland)
5	Application mode:	CAMAG Automatic TLC Sampler III
6	TLC Chamber:	CAMAG, AMD 2 automatic developing chamber
7	Development mode:	Ascending
8	Scanning:	CAMAG TLC scanner 3 with Cats software
9	Experimental conditions:	Temperature 25 $\pm$ 2 $^{\circ}$ C, relative humidity 40 %

HPTLC method, and the selected solvent system toluene: ethyl acetate: diethyl amine (70:20:10) was found suitable for quantitative analysis. Analysis shows that total no of spot was seven, respective R<sub>f</sub> (0.14, 0.24, 0.32, 0.43, 0.51, 0.62) Maximum peak height (17.4, 12.8, 330.1, 47.0, 334.1, 49.0, 18.9) and Peak area (%) (1.69, 0.94, 33.76, 5.11, 47.15, 7.57, 3.77). For quantitative analysis through HPTLC techniques, optimization of solvent system was done using combination of solvent system of varying polarity and the most suitable solvent system was found to be toluene: Ethyl acetate: Diethyl amine (70:20:10). Quantitative analysis was performed through HPTLC techniques using strychnine and brucine as standard marker compound in the *Strychnos nux-vomica*. Content of strychnine and brucine were found to be 4.75% and 3.91%. HPTLC photograph of standard strychnine and brucine and *Strychnos nux-vomica* extract were presented in the Figure 1. The respective HPTLC chromatogram of strychnine and brucine and *Strychnos nux-vomica* extract were presented in the Figure 2 and Figure 3. In the present analysis calibration curve of standard strychnine ( $y = 689.63x + 1006.3$ ,  $R^2 = 0.998$ ) and brucine ( $y = 602.48x + 701.92$ ,  $R^2 = 0.9962$ ) were found to be linear, which was presented in the Figure 4 and Figure 5. The interpretations of result were done using standard calculation and the results suggest that the sample contained considerable amount of strychnine and brucine.

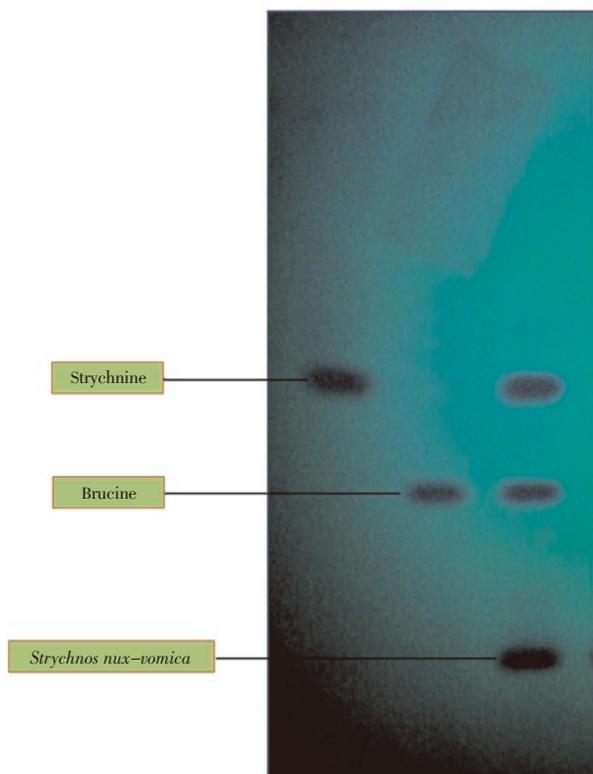


Figure 1: HPTLC photograph of strychnine, brucine and *Strychnos nux-vomica* extract

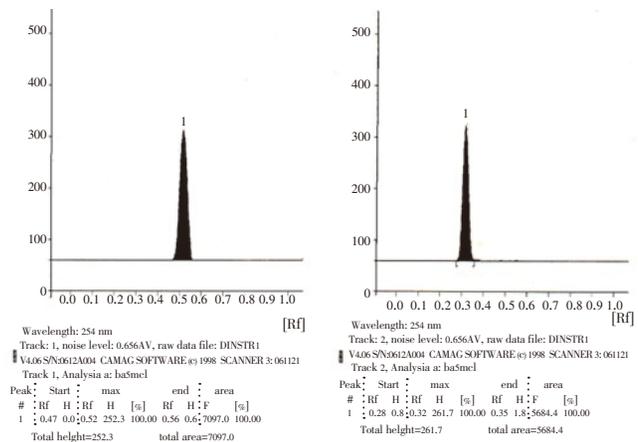


Figure 2: HPTLC Chromatogram of standard strychnine and brucine.

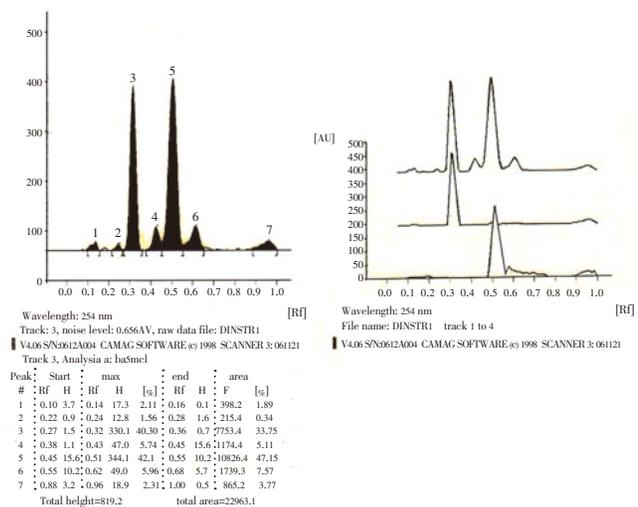


Figure 3: HPTLC Chromatogram of *Strychnos nux-vomica* extract and comparative graph.

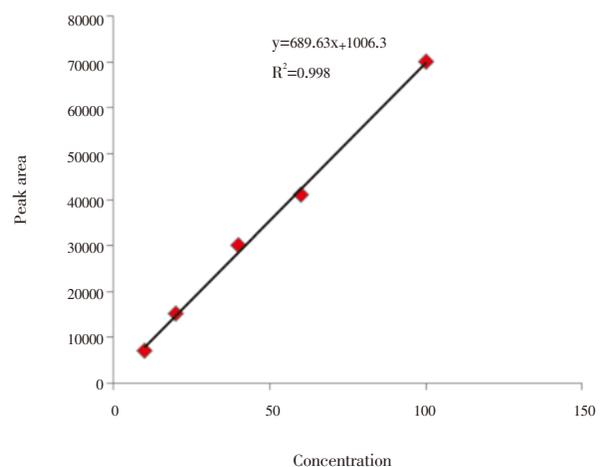
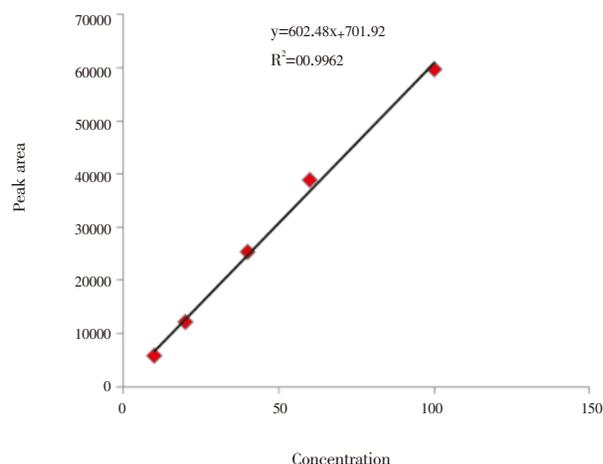


Figure 4: HPTLC calibration curve of standard strychnine.



**Figure 5:** HPTLC calibration curve of standard brucine.

#### 4. Discussion

Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These characters were also used to detect the adulterants if any [13]. In traditional practice, decoction or infusions of medicinal plants are usually made with either alcohol or water as the solvent, which impart marked difference in phytochemical and pharmacological profile of alcoholic and aqueous extracts. Therefore, considering its reported traditional use, hydroalcoholic extracts were taken in the present investigation. The curative properties of medicinal plants are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc [14].

The physico-chemical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The content of strychnine and brucine in *Strychnos nux-vomica* was the high. The presence of flavonoid, tannin, saponins, alkaloid and glycoside in the plants investigated indicated that they could be used in the treatment of burns, wounds and justifies their therapeutic actions, which could be used in drug formulation. The phytochemical analysis revealed that the plants contain bioactive substances, these agents are alkaloids, saponins, tannins, flavonoids, and glycosides. Flavanoids and phenols have been reported to exert multiple biological effects such as anti-inflammatory, anti allergic, antioxidant, antidiabetic, aldose reductase inhibitory potential, anti-viral and anti-cancer activities. Herbs having tannins are astringent in nature and are used for the treatment of diarrhea and dysentery [15–19]. The presence of saponins supports the fact that *Strychnos nux-vomica* has cytotoxic effects such as permeabilization of the intestine as saponins are cytotoxic. Alkaloids are the most efficient therapeutically significant plant substance. Pure isolated alkaloids and the synthetic

derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties [20].

In recent years attention has been drawn to the health promoting activity of plant foods and its active components. Phytoconstituents obtained from natural sources have been gaining importance in the day by day because of the vast chemical diversity. Demands of herbal medicines have been increased in the last two decades, so there is need to ensure the quality, safety and efficacy of herbal drugs [21–23]. Phytochemical standardization is one of the tools for the quality assessment, which includes preliminary phytochemical screening, HPTLC fingerprint analysis and Quantitative analysis of marker compound using modern analytical techniques. In the last few decades (HPTLC) has become known as an important tool for the qualitative semi-quantitative and quantitative phytochemical analysis of herbal drugs and formulations. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of marker compound and mobile phase with very less time [24]. The linear regression curve showed a good linear relationship for strychnine and brucine. Standard strychnine and brucine showed single peak in HPTLC chromatogram. After development the plate was scanned at 254 nm. The calibration curve was prepared by plotting the concentration of strychnine and brucine versus average area of the peak. The amount of strychnine and brucine were quantified using calibration curve.

#### 5. Conclusion

By this method we can interpretate that the *Strychnos nux-vomica* contained considerable amount of Flavonoids, strychnine and brucine. This analytical method can be utilizes for the simultaneous estimation of strychnine and brucine in the *Strychnos nux-vomica*. In future this study this study will be helpful for the quantitative determination of phytoconstituents in *Strychnos nux-vomica*.

#### Conflict of interest statement

The authors report no conflict of interest.

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