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Review Article

COMPREHENSIVE REVIEW ON NUX VOMICA PLANT: SPECTROSCOPIC, CHROMATOGRAPHIC AND HYPHENATED TECHNIQUES

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ABSTRACT

As pharmaceuticals, supplementary and alternative treatments, food supplements, cosmetics, and, more surprisingly, medical devices, medicinal plants have been utilized for a very long time to promote human health. Both conventional and modern medical systems derive their raw materials from medicinal plants. The quality attributes of the herbal materials in the herbal medicines are assessed using a variety of chemical and phytochemical tests, analytical methods, and hyphenated methods. The increasing number of issues in natural product research cannot be solved by using conventional analytical techniques. It is possible to obtain comprehensive chemical profiles of herbal extracts, medicines, and formulations using spectroscopic, chromatographic, and hyphenated techniques. This review aims to detail various analytical techniques for quality control of herbal plant especially for Nux vomica plant.

Keywords: Medicinal Plant, *Strychnos nux-vomica* L, Chemical fingerprints, Strychnine, Brucine.

INTRODUCTION

The Indian medical system includes those believed to have originated in India or those that came to India from other countries and were integrated into Indian culture. These diverse indigenous plant-based systems have been categorised by Indian phytotherapists into six groups: Naturopathy, Yoga, Unani, Homoeopathy, Ayurveda, and Siddha. Over time, each system gained significance and demonstrated advantages for human health (Ravishankar *et al.,* 2008; Akram *et al.,* 2021). Among the numerous Indian systems of medicine, Ayurveda is the most popular and has been practised throughout history (Jaiswal and Williams, 2017). More people are utilising medicinal plant products in the healthcare system recently, all around the world (Bijauliya *et al.,* 2017). Herbal medicine is currently gaining a lot of attention as a complementary therapy useful for both the prevention and cure of lifestyle-related illnesses. It was traditionally practised by surgeons and doctors (named Bheshaja or vaidya) and aimed to promote a healthy and long life rather than treat diseases (Shivatare *et al.,* 2013). The public's interest in complementary and alternative medicine has developed as a result of the escalating negative effects of synthetic drugs, the absence of effective therapies for many chronic ailments, the high cost of new medications, microbial resistance, emerging disorders, etc (Chauhan *et al.,* 2015).

Due to their use in modern medications, nutraceuticals, dietary supplements, pharmaceutical intermediates, traditional medicines, and chemical components for

synthetic drugs, plants have been gaining a lot of interest (Nafiu *et al.,* 2017). Indian heritage is diverse and a rich source of traditional remedies, many of which are derived from plants (Parkash *et al.,* 2018). One of the most significant sources of medications comes from plants. Plants have been used as remedies from the prehistoric era. The Rig Vedic mention of the therapeutic effects of some herbs in India is believed to be the first record of the medicinal use of plants in India. Traditional medical practises and current medical practises are the two main types of health care that primarily employ medicinal plants (Mazid *et al.,* 2012).

Due to the bioactive phytochemicals they contain, medicinal plants are vital to human life and the maintenance of good health. It is common to employ medicinal plants to treat infections, and numerous natural products are utilised as phytotherapies to treat a variety of illnesses (Bhargava *et al.,* 2021). Synthetic drugs controls perform perfectly with clearly established analytical parameters. However, when quality issues are taken into account, herbal products face a variety of special issues. These are as a result of the complex mixes of many secondary metabolites that make up the herbal constituents included within, which can vary greatly depending on external as well as internal variables (Sahil *et al.,* 2011). The quality of natural medicines must be verified due to the rising demand for them. Almost 80% of people use herbs for healing, prevention, and therapy. Therefore, different instruments and techniques must be used to determine and ensure the quality required for introduction into herbal ingredients and products (Balekundri and Mannur, 2020).

Strychnos nux vomica Linn (Family: Loganiaceae) is a poisonous plant of pharmacological significance with a number of medicinal and clinical purposes (Maji, 2017). This species can be found frequently in moist deciduous and semi-evergreen forests throughout the pantropics (Behera *et al.,* 2017). The major component of the plant's traditional medicines is the seed of *Strychnos nux-vomica* L., commonly known as Semen Strychni, Nux vomica, or Ma qian zi (Chinese). Nux vomica is a traditional remedy used by Ayurvedic physicians to treat a wide range of ailments including upset stomach, nervous system problems, chronic rheumatic diseases, urinary incontinence, sexual dysfunction and rejuvenation (Guo *et al.,* 2018). Pharmacologically, *Strychnos nux-vomica* has shown anticancer, antibacterial, anti-inflammatory, antioxidant and antiphagic effects. Additionally, their specific effects on the cardiovascular system, blood sugar level, bone cells, and brain system have been researched (Patel *et al.,* 2012). The official component in traditional pharmacopoeias is nux-vomica seed. This plant is abundant in flavonoids, alkaloids, triterpenoids, tannins, glycosides, lignins, and steroids. Strychnine and brucine are the two main poisonous alkaloids that have been isolated from various portions of nux vomica out of more than 90 different chemical substances. They can be found in fruit pulp, wood, bark, roots, seeds, and hard fruit shells in addition to seeds. 2.6% to 3% of the total amount of alkaloids are found in Indian nux-vomica seeds, out of which 1.25 to 2.5% are strychnine & 1.5 to 1.7% are brucine. Additionally, the seeds include a glycoside referred to as loganin, 3% fixed oil, and chlorogenic acid. Two of

Strychnos nux-vomica's most potent and pharmacologically active phytoconstituents are strychnine and brucine. These two substances stimulate the central nervous system but are fatal when consumed in large doses (Patel *et al.,* 2017).

Starting from the source of the plant material, quality monitoring of the medicinal plants is essential. Numerous environmental elements, including geographic location, soil quality, temperature, rainfall, and others, can affect the phytochemical composition of plant material and the consequent quality. Composition can also be impacted by taxonomy, collecting time, collection method, cultivation, drying, harvesting and storage conditions, and preparation and processing techniques. Poor quality final products can also result from contamination by bacteria, chemical substances like pesticides and heavy metals, as well as by insects and animals, throughout any of these stages. To comply with the current requirements of quality, safety, and effectiveness, standardisation of all these aspects is required (Sahil *et al.,* 2011). Standardisation of herbal raw pharmaceuticals involves collecting passport information on the plants used, authenticating the plants by microscopic and molecular analysis, identifying the chemical makeup of the drugs using various chromatographic techniques, and testing the whole plant's biological activity (Nikam *et al.,* 2012). For effective standardization, it is crucial to use precise and rapid modern analytical techniques to assess the quality, efficacy and purity of herbal plants. The most popular methods for determining the quality of medicinal herbs and the products derived from them are chromatography & it's hyphenation with spectroscopic techniques (Sharma and Yadav, 2023).

For the purpose of quality control of herbal remedies, chemical fingerprints produced by chromatographic methods, particularly hyphenated chromatography, are highly advised because they may accurately represent the "chemical integrities" of natural remedies & can be used for both product identification and verification. For qualitatively identifying small levels of pollutants, High Performance Thin Layer Chromatography and Thin Layer Chromatography are helpful approaches. For quality assurance and standardization, other analytical techniques are also often utilised, including Volumetric Analysis, Gravimetric Determinations, Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Column Chromatography (CC) and Spectrophotometric Methods (Patel and Patel, 2016). Due to the NMR and MS techniques' ever-improving capabilities, their importance is still growing. Although its use in drug quality control is declining, UV spectrophotometry is still one of the most used techniques for analysing both bulk medications and drug formulations. While atomic spectroscopy is crucial in the identification of metal contaminants, IR & near-infrared (NIR) spectroscopies are crucial techniques for fast identification of pharmaceuticals. High-Pressure liquid chromatography-mass spectrometry (HPLC-MS), High-Pressure liquid chromatography-nuclear magnetic resonance spectrometry (HPLC-NMR), Capillary electrophoresis-diode array detection (CEDAD) and High-Pressure liquid chromatography-diode array detection (HPLC-DAD) are recent methods of

applying hyphenated chromatography and spectrometry (Mondal, 2018).

I. SPECTROSCOPIC METHODS:

Indrani Chakraborty et al (2021) developed used Fourier Transform Infrared Spectroscopy to study differences in free and bound water molecules in various homoeopathic potencies. This article looks at the FTIR spectra of several homoeopathic potencies with fixed ethanol contents to determine if there are any variations in free water molecules. When compared to water, HWHM was 22 cm-1 narrower in the case of Nux Vom 8cH. In the example of Nux Vom 8 cH, the HWHM was 8 cm-1 narrower than the water.

Muhammad Rahil Aslamet al (2022) utilised in-vitro preliminary phytochemical analysis method compare the anti-arthritic and antiphlogistic effects of various traditionally used herbal plants. Dried Nux-vomica extracts were placed within a spectroscope (Shimadzu, Japan) with a 400–4000 cm1 scanning range and a 4 cm resolution. Alkaloids, anthraquinones, saponins, terpenoids, and phenols were found in all of the extracts by phytochemical screening, while aromatic or aliphatic compounds, organic acids, amine group esters, halogens, phenolics, and organic acids were found using FTIR.

Jaya Guptaa et al (2016) reported *Strychnos nux-vomica* L. contains flavonoids that have been isolated, detected, and their antioxidant capacity analyzed. UV spectrum: showed two prominent absorption peaks around 355 and 258 nm. Mass spectroscopy: Molecular ion m/z 610 [M+] of an isolated molecule with the chemical formula C27H30O16 is detected. IR Spectrum: Along with C-OH vibration at 1384 cm^{-1},CO group at 1462 cm^{-1}, CH-bending at 2715 cm^{-1} and CH2 stretching at 2842 cm^{-1}, OH stretching was seen in the IR spectra at 3410 $cm^{-1}$and 3322 cm^{-1}.

Michel Frederich et al (2004) reported analysis of extracts of *Strychnos nux-vomica*, Strychnos icaja, and Strychnos ignatii metabolomically using 1H NMR and multivariate analysis methods. Utilizing 1H nuclear magnetic resonance spectroscopy (NMR) and multivariate analysis techniques, three metabolic profiles of Strychnos - *Strychnos nux-vomica* (root, bark, seed, stem bark), Strychnos ignatii (seed), Strychnos icaja (root bark, collar bark, Leaf, stem bark) were determined.

Marie-Caroline Jonville et al reported (2013) utilizing spectrometric data, strychnochrysine and three novel bisindole alkaloids were obtained and recognized. They showed in this study that it is simple to deduce a stereochemical change in these alkaloids using 13C HSQC NMR data. Reverse-phase column chromatography was used to purify the extract of monoindole alkaloids. Centrifugal partition chromatography (CPC) was used to separate the fractions containing orange alkaloids.

II. Chromatography methods:

S. P. Dhanabal et al (2012) reported phytochemical evaluation and standardization of Nux-vomica extract by HPTLC method. The extraction procedure aided by the use of methanol. With the help of toluene: Ethyl acetate: Diethyl amine (70:20:10) as mobile phase, Strychnine and brucine contents were found to be 4.75% and 3.91%, respectively.

Abid Kamal et al (2012) developed High Performance Thin Layer Chromatography (HPTLC) for simultaneous estimation of strychnine and brucine in Nux-vomica seed.

The technique utilised high performance TLC to resolve the sample and simultaneous measurement of strychnine and brucine. In the Soxhlet apparatus, a precisely defined quantity (10 g) of Nux-vomica seed powder was extracted with chloroform. Using the mobile phase Chloroform: Methanol : Formic acid (8.5:1.5:0.4), the Rf values of strychnine and brucine were found to be 0.60 and 0.69, respectively.

Dr. D. A. Shanbhag et al (2008) developed High Performance Thin Layer Chromatography (HPTLC) for the standardization of a homoeopathic mother tincture of Nux Vomica. Different ingredients in nux vomica tincture have Rf values of 0.16–0.18, 0.27–0.30, 0.31–0.32, 0.38–0.40, and 0.74 using toluene:Acetate : ethanol As the mobile phase, diethyl amine (7:2:1).

Shakila Ramachandran et al (2022) reported that *Strychnos nux-vomica* nuts can be subjected to quantitaive analysis, High Performance Thin Layer Chromatography finger print analysis, and several spectrometric analyses. The S. nux-vomica seeds were collected, identified pulverised, and contributed to finger print profiling by HPTLC, visualisation of thin layer chromatographic separation, and proximal analytical parameters. On the HPTLC plate, two spots were produced by Dragendorff's reagent, and seven spots were obtained by derivatizing vanillin sulphuric acid (VSR) at 254 and 366 nm, respectively. After derivatization in HPTLC, the results revealed 12 peaks in 254 nm, 9 peaks in 366 nm, and 7 peaks in 520 nm.

Achu Hashim et al (2015) developed analysis using RP- high pressure liquid chromatography can be used to determine the amount of a toxin in Strychnos nux vomica. After a preliminary phytochemical examination of *Strychnos nux-vomica* seeds, TLC used to validate the identification of the phytochemicals. 6-gingerol was separated using a C18 - ODS (Octadecylsilane), Lichrospher Reverse Phase 18e (5 m)column with a UV-visible detector at 254 nm and a mobile phase comprising methanol: Water (55:45).

Shabnam Anjum Ara et al (2021) reported that acute oral toxicity of habb-e-azaraqi that is conventional unani formulation based on nux-vomica, was assessed using TLC, HPTLC fingerprinting. The formulation of Habb-e-Azaraqi was examined using current technical standards and exposed to substantial and scientific standardization criteria, such as TLC and HPTLC analysis, which supported the formulation's identity and purity. Toluene:ethyl acetate (8:2, v/v) was used as the mobile phase & alcoholic extracts were applied to TLC plates, spotted and developed on silica gel 'G' plates.

Ingole Nirwan et al (2021) reported that Strychnine, a plant alkaloid, can be analysed by TLC employing a new solvent system in hazardous baits. Thin layer chromatography was used in the investigation to design a solvent system for measuring the concentration of strychnine in hazardous baits. This solvent system will be useful for identifying strychnine in cases of animal poisoning since it employs the ratios 70:20:10(v/v) of Dichloromethane: Methanol: Petroleum as the mobile phase.

J. Bandopadhyay et al (1997) reported that *Strychnos nux-vomica*'s vegetative parts exhibit seasonal variations in strychnine and brucine content. *Strychnos nux-vomica*'s

various vegetative sections were examined using HPLC to identify differences in strychnine and brucine. The root bark that was harvested in December showed the highest concentrations of strychnine and brucine, with concentrations of 5.4 mg/g dry weight and 10.12 mg/g dry weight, accordingly.

Rajashree Mashru et al (2017) reported RP-HPLC technique for the detection of strychnine in formulations containing nux vomica has been developed and validated. HPTLC with a UV detector was developed to identify active alkaloids such as strychnine in homeopathic medicines. The mobile phase for the RP-High pressure liquid chromatography separation was Acetonitrile: Phosphate buffer(55:45) pH3.6 with 0.2% TEA and 0.02% W/V SLS using a Bischoff-chrombudget C18 (250mm 4.6mm x 5m) column.

Baochang Cai et al(2014) reported pharmacological analysis of Nux Vomica's total alkaloids after reducing the strychnine conent. The study's goal was to determine whether it was possible to get improved total alkaloid fraction (TAF) by reducing the amount of strychnine TAF that was extracted from nux vomica after processing. The ratios of brucine to strychnine for TAF and MTAF were found to be 1:2.05 and 2.2:1, accordingly, and Total Alkaloid Fraction was shown to be near 3.17 times more dangerous than MTAF. With the aid of a Shimadzu High Pressure Liquid Chromatography system (Kyoto, Japan), which featured an LC-20AT pump and an SPD-20A UV-Visible detector, strychnine and brucine were evaluated simultaneously in TAF or MTAF.

Xiao Wang et al (2013) reported that *Strychnos nux-vomica* can be separated into strychnine and brucine on a large scale using counter-current chromatography with pH zone refinement. For the first time, preparation-stage counter-current chromatography was used to effectively separate a raw extract of *Strychnos nux vomica* into strychnine and brucine. According to HPLC results, these compounds have purities of 95.1% and 97.0%, respectively. An electron ionization-mass spectrometer (EI-MS) and proton nuclear magnetic resonance spectrometer were used to determine the structures of the separated compounds.

P. Miao et al (1998) reported Strychnine is separated and purified using high-speed countercurrent chromatography from the raw extract of *Strychnos nux-vomica*. HSCCC is a remarkable method for purifying natural products. On TLC, authentic pure chemicals were used to identify fractionated components. HPLC, IR, and FTMS analyses were also performed.

Ghasem Haghi et al (2010) reported that Hydrophilic-interaction chromatography and UV detection were used to analyze strychnine and brucine in raw and treated *Strychnos nux-vomica* seeds. Strychnine and brucine, the two main alkaloids found in Nux-vomica Linn. plant nuts and preparations, have been determined by hydrophilic interaction liquid chromatography, which has been developed and validated Acetonitrile-water 90:10 (v/v) was used as the mobile phase along with a flow rate of 1.0 mL/min for separation on a CARBOSep column. Average strychnine and brucine recoveries from spiked samples were 99.9% & 98.5%, respectively.

III. Hyphenated techniques:

Jinwoong Kim et al (2004) developed liquid chromatography-electrospray mass spectrometry (LC-ESI-MS) for the estimation of strychnine from detoxicated Strychno nux-vomica seeds. The HPLC-ESI/MS technique utilised in the present investigation to determine the amount of strychnine in the detoxifying Strychno nux-vomica seeds. The base fragment ion peak at m/z 264 [M + H C3H5NO]+ was seen in the m/z 335 [M + H]+ MS/MS spectra of untreated and detoxified Strychno nux-vomica seeds.

PN Prasad Maddisetty et al (2017) used HPLC and GC-MS for recognizing and determining the amount of brucine and strychnine in Nux-vomica leaves. The objective of current study was to evaluate this plant's phytochemical composition as well as amounts of brucine and strychnine.. Bioactive compounds were discovered utilizing the GC-MS and NIST Library. Strychnine and brucine were determined by High Pressure Liquid Chromatography.

Yao Shen et al (2013) reported quicker and more environmentally friendly alkaloid analysis, hyphenation of improved microfluidic preparation of samples with nano -LC . With the pretreatment, separation, and identification of samples integrated into one system, manpower costs and solvent use are greatly reduced while analysis is sped up. The method was applied to a real sample made up of *Strychnos nux-vomica* seeds. Strychnine used as the system to assess the efficacy of the improved 3-phase chip's extraction.

Suglanthy M et al (2020) reported developed Gas chromatography–mass spectrometry chemical analysis of Nux-vomica L. leaves for for their biopesticidal activities. Solvent-based extraction of *Strychnos Nux-vomica* leaves with ethanol and methanol revealed the most important bioactive chemicals according to Gas chromatography–mass spectrometry (GC-MS) analysis. In the GC-MS analysis of methanolic and ethanolic leaf extracts, 44 chemicals were found.

Chunjie Wu et al (2014) reported that *Strychnos Nux-vomica* dried out in sands is being monitored online, and its chemical composition is being analyzed using UPLCLTQ-Orbitrap-MS. On the other hand, Ultra Performance Liquid Chromatography-Linear Trap Quadrupole-Orbitrap MS is an effective and quick technique for chemical profiling in both raw and treated nux-vomica. ONTMS methods are more precise for parching nux-vomica in sands. By using HPLC-DAD, 14 significant compound from raw and processed nux-vomica were discovered at once

Tang HB et al (2013) reported detailed quality control of Semen Strychni using the High-Pressure Liquid Chromatography with Diode-Array Detection method(HPLC-DAD). For the simultaneous identification of loganin, chlorogenic acid, and four Strychnos alkaloids, a selective HPLC-DAD approach has been developed. In order to simultaneously determine the amounts of the six chemicals in the collected samples of seeds coat, seed leaf, endosperm, and entire seed, the developed HPLC-DAD method was utilised. The results demonstrate that the High-Pressure Liquid Chromatography in hyphenation with Diode-Array Detection method is an effective & comprehensive technique for Semen Strychni quality control.

Guo Y et al (2022) developed UPLC-MS/MS to estimate the amount and release properties

of six compounds in various stages of the "Glycyrrhiza glabra-Nux vomica" decoction. An Ultra performance liquid chromatography - tandem mass spectrometer (UPLC-MS/MS) method has been developed and validated for the simultaneous quantitative analysis of brucine, strychnine, liquiritin, isoliquiritin, and glycyrrhizic acid concentrations in "Glycyrrhiza glabra-Nux vomica" decoction. The outcome of the content determination reveal that the sedimentary phase's most hazardous strychnine comprised 75.70% of all the components.

CONCLUSION

The standardizing process ensures the quality and uniformity of the therapeutically effective plant materials. Analytical techniques are used to identify and separate the different compounds present in herbal plants. India should research the medicinally significant herbs. Only if the herbal plants are assessed and examined utilizing advanced modern standardization methods, such as chromatographic, spectroscopic, and other hyphenated approaches, can this be accomplished. The pharmaceutical industry as a whole will profit from the use of high-technology oriented sophisticated hyphenated procedures as a quick and unambiguous instrument for herbal research.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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