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Research Article

INTEGRATED PHYTOCHEMICAL ANALYSIS OF STRYCHNOS NUX-VOMICA USING MOLECULAR SPECTROSCOPY (ULTRAVIOLET-VIS AND FOURIER TRANSFORM INFRARED) AND HYPHENATED CHROMATOGRAPHY (GAS CHROMATOGRAPHY-MASS SPECTROMETRY)

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ABSTRACT

Objectives: Integrating ultraviolet (UV)-Vis, Fourier transform infrared (FTIR), and gas chromatography-mass spectrometry (GC-MS) techniques provides a comprehensive approach to phytochemical analysis by combining compound quantification (UV-Vis), molecular identification (FTIR), and detailed component profiling (GC-MS). The objective of this study was to analyze the phytochemical content of the methanolic leaf extract of *Strychnos nux-vomica* L.

Methods: The photochemical content of the methanolic leaf extract was analyzed by molecular spectroscopic techniques, i.e., UV-Vis and FTIR, along with a hyphenated chromatography, i.e., GC-MS.

Results: In the UV-Vis spectrum, the most prominent peaks are in the UV region (200–300 nm range), with the highest absorbance at 204.0 nm (0.583371) corresponding to phenolic compounds. The peaks at 225 and 354 aligns with anthraquinones, and 259 may correspond to phenylpropanoid. Several smaller peaks are also visible in the 330–371 nm range. In the FTIR spectrum, 11 distinctive bands were absorbed, i.e., 3375.88, 2925.63, 2361.21, 1710.59, 1607.42, 1452.60, 1377.41, 1266.64, 1073.04, 1029.52, and 669.71: A broad peak at 3375.88 cm^{-1} which correspond to phenolics, flavonoids and alkaloids and a narrow peak at 669.71 cm^{-1} which correspond to aromatic compounds (alkaloids and flavonoids). The GC-MS chromatogram identified eight major phytochemicals according to their retention times, peak areas, and molecular characteristics. Based on the peak area, the highest and lowest peaks observed are at 9.324 min and 27.96 min, with areas of 14.7% and 2.96%, respectively. The peak at 9.324 min was identified as 2-butenethioic acid, S-[2-(acetylamino)ethyl] ester with 14.7% area, and the peak at 27.96 min was identified as tridecanoic acid, a methyl ester with 2.96% area.

Conclusion: The integration of UV-Vis, FTIR, and GC-MS techniques provided a comprehensive phytochemical profile of *S. nux-vomica* methanolic leaf extract. The results confirmed the presence of multiple bioactive compounds, supporting the plant's traditional medicinal applications. These findings lay the groundwork for future research that should focus on isolating these compounds and validating their pharmacological potential through biological and toxicological studies.

Keywords: *Strychnos nux-vomica*, Phytochemicals, Ultraviolet-Vis, Fourier transform infrared, Gas chromatography-mass spectrometry, Secondary metabolites.

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INTRODUCTION

Plants and their derived substances have been an integral part of traditional health-care systems for millennia. They have played a pivotal role across different cultures, offering a rich source of bioactive compounds with potential therapeutic applications [1,2]. Among them, *Strychnos nux-vomica* L., native to Southeast Asia and India, is commonly known as the poison nut/Kupilu/Quaker button or the *nux-vomica* tree, and has significant medicinal value, primarily attributed to its alkaloids [3,4]. The phytochemical composition of *S. nux-vomica* has been extensively studied due to its diverse pharmacological properties. Alkaloids (brucine and strychnine), flavonoids, terpenoids, glycosides, and phenolic compounds are the main bioactive components; many of these substances have strong anti-inflammatory, antioxidant, antibacterial, and neurostimulant properties [5-8]. Despite their toxic nature, controlled formulations of *S. nux-vomica* extracts have been used in Ayurvedic, Unani, and Chinese medicine for treating neurological disorders, rheumatism, fever, and digestive ailments [9,10].

However, medicinal plants harbor a complex matrix of compounds that are susceptible to variations induced by environmental factors and manufacturing conditions, necessitating sophisticated analytical techniques for quality control [11]. Environmental factors, geographical location and climate, manufacturing conditions, planting and harvesting schedules, plant maturity at harvest, and storage procedures are some of the factors that affect the consistency of plant extracts [12].

Consequently, thorough analysis of plant extracts necessitates an integrated approach, leveraging techniques such as molecular spectroscopic (ultraviolet [UV]-Vis spectroscopy and Fourier transform infrared [FTIR] spectroscopy) along with hyphenated chromatographic (gas chromatography-mass spectrometry [GC-MS]) to provide a comprehensive understanding of their phytochemical composition [13-16]. The characterization of extracts, which involves identifying their chemical components, is essential for assessing their overall quality and biosafety [17].

UV-Vis spectroscopy, by identifying their electronic transitions, helps identify flavonoids, alkaloids, and phenolic chemicals and offers

important insights into the presence of conjugated systems in plant metabolites [18]. FTIR spectroscopy, on the other hand, is a powerful and non-invasive tool for identifying phytochemical functional components such as hydroxyl, carbonyl, and amine groups, which play crucial roles in the bioactivity of plant-derived compounds [19-21]. GC-MS analysis is particularly effective in profiling volatile and semi-volatile secondary metabolites by providing detailed structural information based on mass fragmentation patterns [22]. Integrating these three analytical techniques, such as spectroscopy, chromatography, and mass spectrometry – enables a comprehensive characterization of phytochemicals, offering more profound insights into their structural composition and potential medicinal properties, and also ensuring the desired quality standards [23].

Thus, the present study aimed to integrate molecular spectroscopic techniques, such as UV-Vis and FTIR, along with a hyphenated chromatographic method, i.e., GC-MS, to analyze the methanolic leaf extract of *S. nux-vomica*. By characterising its phytochemical composition, functional groups, and major bioactive compounds, this study sought to validate its traditional medicinal claims and explore its potential pharmacological applications. In addition, these findings may contribute to our future toxicological and pharmacokinetic studies, ensuring the safe therapeutic use of *S. nux-vomica* in modern medicine.

METHODS

Plant samples

The leaves of the selected plant were collected from Chengalpattu district, Tamil Nadu, India, and subsequently identified. The herbarium has been deposited in the PG and Research Department of Botany, Pachaiyappa's College, Chennai, Tamil Nadu.

Chemicals and reagents

The analytical-grade chemicals and reagents utilized in this investigation were bought commercially from HiMedia Laboratories Pvt. Ltd. in Mumbai, India.

Preparation of crude extraction of the plant

The collected leaf samples were allowed to dry at room temperature in the shade, and after drying, it was pulverized. The preparation of crude extraction was performed according to the method described by [24], with slight modifications. In brief, 25 grams of pulverized material was placed in a 500 mL conical flask containing 225 mL of methanol. At room temperature, the flask was shaken for 72 h at 250 rpm. Filtration was done on the resultant mixture. To create concentrated extracts, the filtrates were vacuum-concentrated at room temperature in a rotating evaporator.

Molecular spectroscopic (UV-Vis and FTIR) analysis

UV-Vis spectroscopy analysis

The methanolic leaf extract of *S. nux-vomica* was subjected to a UV-visible spectrophotometer (the Jasco V-730 UV-VIS model with silicon photodiode detectors in a range of 190–1100 nm) to detect the presence of conjugated systems in the plant extracts. With a 1.0 nm bandwidth and a 0.5 nm data interval, the absorbance was measured between 800 and 200 nm.

FTIR spectroscopic analysis

To identify functional groups linked to various phytochemicals, the methanolic leaf extract of *S. nux-vomica* was subjected to an attenuated total reflection-Fourier transform-infrared spectroscopic analysis using the Jasco FT/IR-4700 spectrometer model with DLaTGS (with Peltier temperature control) detectors. With a nominal resolution of 4 cm^{-1}, spectra were captured within the measuring range of 4000–400 $cm^{-1\,nm}$.

Hyphenated chromatographic (GC-MS) analysis and identification of components

The methanolic leaf extract of *S. nux-vomica* was subjected to GC-MS analysis to detect major bioactive compounds and confirm the phytochemical complexity of *S. nux-vomica*. Hewlett-Packard (HP) 6890/5973 GC-MS with Agilent 7890A/5975 C GC HP-5, running at 1000 eV ionization energy was used along with capillary column (phenyl methyl siloxane, 25 m × 0.25 mm i.d.) through carrier gas of helium (He of 0.9 mL/min) with split ratio of 1:5. The oven temperature was regulated between 80°C (2 min) and 280°C at a rate of 1–40°C/min, whereas the detector temperature was set between 250°C and 280°C. Using the split ratio and mass scan of 50–600 amu, 2.0 µL of the corresponding diluted samples was manually injected in the splitless mode. The GC-MS runs for 50 min in total, and the peak area percentage normalization represents the relative percentage of each extract element.

The National Institute of Standards and Technology (NIST) database and the Wiley Library for mass spectra were used to analyze the GC-MS mass spectrum. The NIST library's collection of known components' mass spectra was compared to the unknown component's spectrum. The components of the test materials were identified by their names, molecular weights (MW), and structures [25].

RESULTS

The present study integrates Molecular Spectroscopic and Hyphenated Chromatographic techniques to analyze the phytochemicals and bioactive compounds in the methanolic leaf extract of *S. nux-vomica*, a plant known for its medicinal properties. The results provide a comprehensive understanding of the phytochemical profile, functional groups, and possible bioactive compounds.

The UV-Vis spectrum (Fig. 1a) exhibits several distinct and complex absorption peaks that span the 200–800 nm wavelength range. Our analysis focused on identifying 10 well-resolved peaks that did not overlap, which were observed at wavelengths of approximately 204, 225, 236.5, 259, 279, 300, 311.5, 343.5, 354, and 364 nm, with corresponding absorbance values that indicated significant electronic transitions in the extract. The most prominent peaks were in the UV region (200–300 nm range), with the highest absorbance at 204.0 nm (0.583371). Several smaller peaks are also visible in the 330–371 nm range. These peaks represent specific electronic transitions in the compounds in the extract and can be used for the identification and characterization of the bioactive compounds in the plant extract. The spectrum exhibits a gradual increase in absorbance from 200 nm to 210 nm, followed by a decline toward longer wavelengths. The peaks between 200 and 350 nm align with alkaloids, flavonoids, and phenolic compounds.

The attenuated total reflectance (ATR)-FTIR spectrum (Fig. 1b) displays a range of characteristic absorption bands that can provide insights into the functional groups and molecular structure corresponding to bioactive compounds in the sample. In total, 11 distinctive bands were absorbed, with a broad peak at 3375.88 cm^{-1} and a narrow peak at 669.71 cm^{-1}. The absorbed bands with their functional groups and possible compounds are tabulated in Table 1. There is a peak at 2361.21 cm^{-1}, which may represent either CO_2 asymmetric stretching, likely due to environmental interference during sample analysis, or C≡N stretching, which may be from nitrile groups in alkaloids, which is strongly supported by another peak at 1029.52 (C-N amine stretch).

The GC-MS chromatogram (Fig. 2a and b), based on the comparison of the mass spectra of each phytochemical with the NIST and Wiley library, characterized and identified eight major phytochemical compounds based on their retention times (RT), peak areas, and molecular characteristics. The chromatogram shows good peak separation and resolution based on the distinct RTs. The majority of the compounds elute within the first 10–11 min, and the major peaks appear in the early RT region (9–10 min), suggesting that they are relatively more polar or volatile compounds. Two later-eluting compounds appeared at significantly higher RTs (27.96 and 47.251 min). Based on the peak area, the highest and lowest peaks observed are at 9.324 min and 27.96 min,

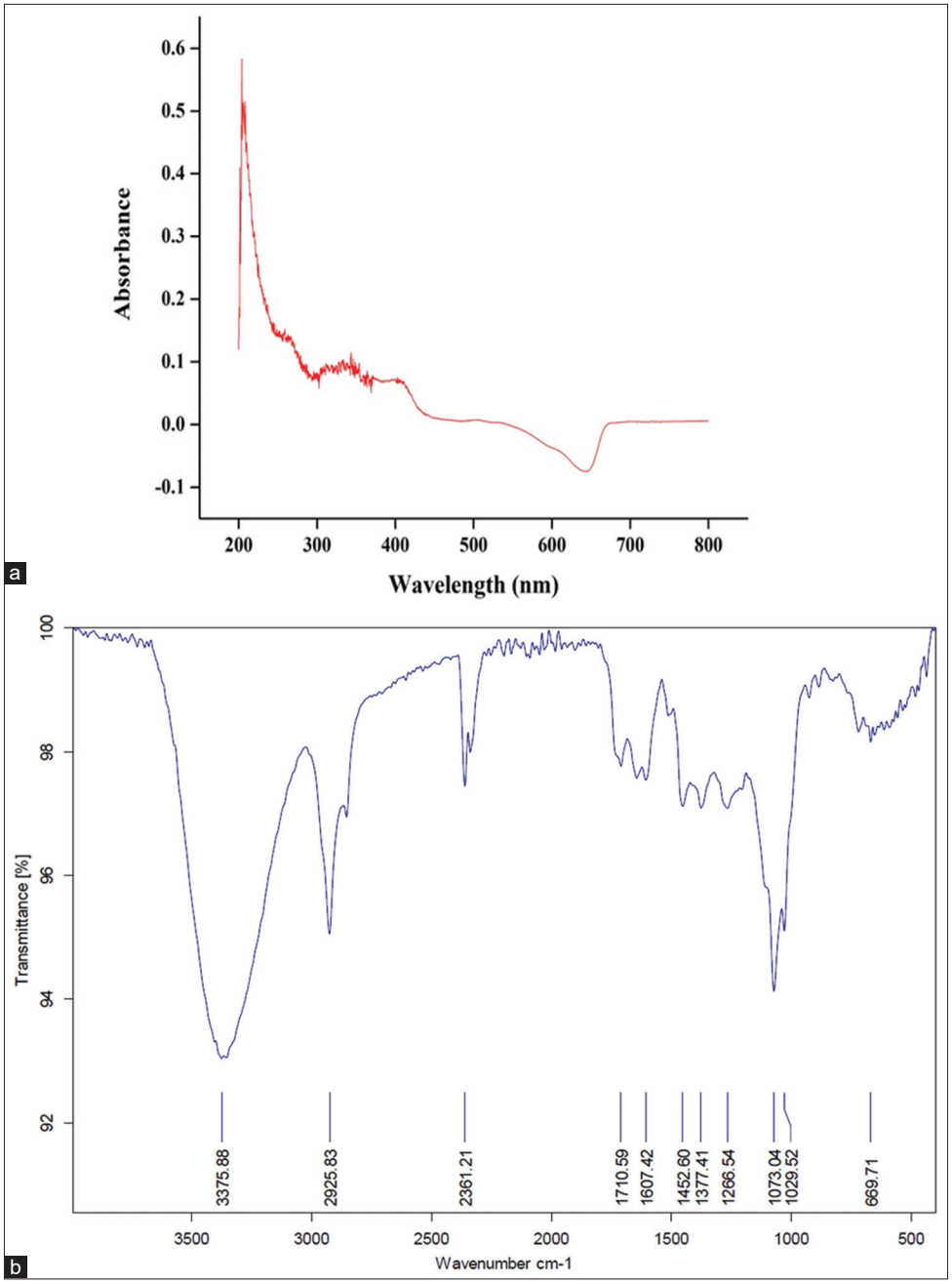


Fig. 1: Molecular spectroscopic analysis of *Strychnos nux-vomica methanolic* leaf extract. (a) Ultraviolet-Vis spectrum; (b) Fourier transform infrared spectrum

Table 1: All peaks, corresponding functional groups, and possible phytochemical class in methanolic leaf extract of *Strychnos nux-vomica*

Peak (cm^{-1})	Functional group	Possible phytochemical class
3375.88	O-H (hydroxyl stretch, strong, broad)	Phenolics, flavonoids, and alkaloids
2925.63	C-H (alkane stretch)	Fatty acids and terpenoids,
2361.21	CO_2 asymmetric stretch (minor) C≡N stretching	Atmospheric interference alkaloids
1710.59	C=O (carbonyl stretch, strong)	Aldehydes, ketones, flavonoids, tannins, and terpenoids
1607.42	C=C (aromatic ring stretching)	Alkaloids, flavonoids, and phenolics
1452.60	C-H bending (CH_2/CH_3)	Terpenoids and alkanes
1377.41	C-O (phenol, ester stretch)	Flavonoids and glycosides
1266.64	C-O-C (ether stretch)	Alkaloids, glycosides
1073.04	C-O (alcohols, ethers)	Sugars and glycosides
1029.52	C-N (amine stretch)	Alkaloids (strychnine and brucine)
669.71	C-H bending (aromatic)	Aromatic compounds (alkaloids and flavonoids)

with area of 14.7% and 2.96%, respectively. The peak at 9.324 min was identified as 2-butenethioic acid, S-[2-(acetylamino)ethyl] ester with 14.7% area, and the peak at 27.96 min was identified as tridecanoic acid, a methyl ester with 2.96% area. The MWs of the identified compounds ranged from 44.1 to 369.8 g/moL. The identified compounds, their retention indices (RT), molecular formulae, molecular structure, MW,

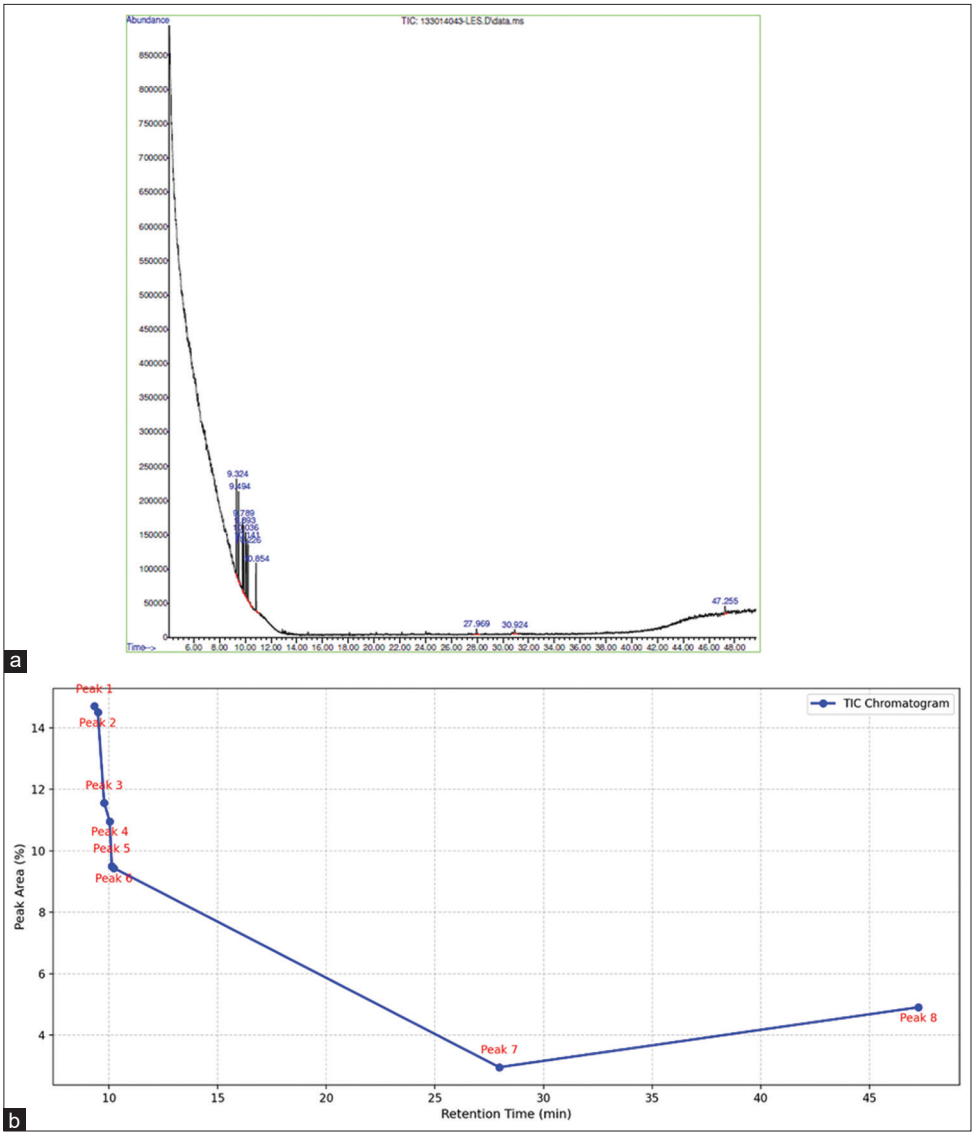


Fig. 2: Gas chromatography-mass spectrometry chromatogram (hyphenated chromatographic analysis) of *Strychnos nux-vomica* methanolic leaf extract. (a) Gas chromatography-mass spectrometry chromatogram (Abundance vs. retention time); (b) total ion current chromatogram (peak area vs. retention time)

percentage composition (area% %), and possible biological activities are shown in Table 2.

DISCUSSION

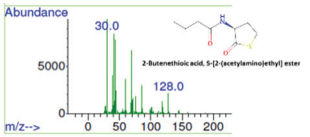
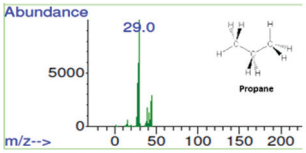
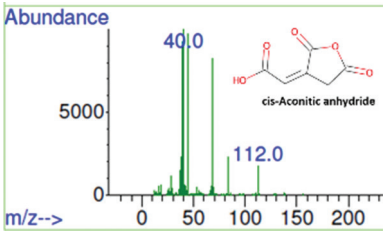
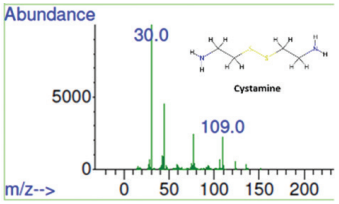
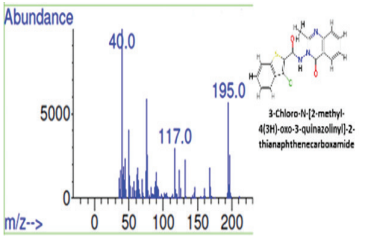
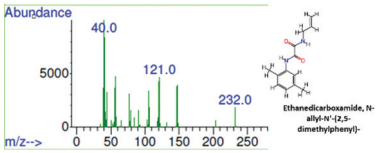
Natural products obtained from plants are known to possess bioactivity. However, it should be properly characterized for standardization, quality control, and post-marketing surveillance of natural products to safeguard the end users [26]. A complete phytochemical characterization involves integrating molecular spectroscopic and hyphenated chromatographic techniques such as UV-Vis, ATR-FTIR, and GC-MS data, respectively. Each technique provides unique insights.

The UV-Vis spectrum provides insight into the possible phytochemicals and identifies conjugated systems such as flavonoids, alkaloids, and phenolics. The UV-Vis spectrum can be used to identify compounds with π-bonds, σ-bonds, lone pairs of electrons, aromatic rings, and chromophores [27].

The various distinct peaks in the UV-Vis spectrum suggest the presence of various chromophoric groups, unsaturated groups, and heteroatoms in the extract [14,27]. For example, the intense absorption observed at approximately 204 nm likely corresponds to strong π→π* transitions, which are typical in conjugated systems or phenolic compounds [28,29]. The absorbance gradually decreases toward the visible region, suggesting that there were fewer chromophores in that range. The additional peaks in the mid-UV range (225–364 nm) imply complex interactions among multiple active compounds, which may include alkaloids, phenolic, and flavonoids, and their derivatives known to be present in *S. nux-vomica* [30]. Particularly, the peaks at 225 and 354 aligns with anthraquinones as previously reported by [31]. The peak at 259 may correspond to phenylpropanoids, which are simple phenolic compounds as earlier reported by [32]. The spectrum strongly indicates the presence of alkaloids, flavonoids, tannins, and phenolic compounds. The weak absorbance in the visible range implies that colored compounds such as anthocyanins or carotenoids are minimal [27]. The observed spectral pattern is consistent with previously reported UV-Vis profiles for similar plant extracts, where multiple absorption bands have been attributed to structurally varied natural products and secondary metabolites, which play a key role in biological activity [14,25,33].

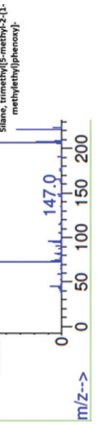
Based on the peak values seen in the infrared radiation spectrum, the FTIR spectrum was utilized to determine the functional groups found in the bioactive components of S. *nux-vomica* leaf samples. It has been shown

Table 2: Phytocomponents identified in *Strychnos nux-vomica* extract

Peak no	Peak RT (min)	Peak area (%)	CAS	Name of compound	Molecular formula	Molecular weight	Mass spectrum
1	9.324	14.7	023784-20-5	2-Butenethioic acid, S-[2-(acetylamino) ethyl] ester	$C_8H_{13}NO_2S$	187.257	
2	9.485	14.51	000074-98-6	Propane	C_3H_8	44.1	
3	9.778	11.56	006318-55-4	cis-Aconitic anhydride	$C_6H_4O_5$	156.09	
4	10.029	10.96	000051-85-4	Cystamine	$C_4H_{12}N_2S_2$	152.3	
5	10.134	9.5	304882-71-1	3-Chloro-N-[2-methyl-4 (3H)-oxo-3-quinazolinyl]-2-thianaphthenecarboxamide	$C_{18}H_{12}ClN_3O_2S$	369.8	
6	10.217	9.44	331864-72-3	Ethanedicarboxamide, N-allyl-N′-(2,5-dimethylphenyl)-	$C_{13}H_{16}N_2O_2$	232.28	

(Contd...)

Table 2: (*Continued*)

Peak no	Peak RT (min)	Peak area (%)	CAS	Name of compound	Molecular formula	Molecular weight	Mass spectrum
7	27.96	2.96	001731-88-0	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228.37	
8	47.251	4.91	055012-80-1	Silane, trimethyl[5-methyl-2-(1-methylethyl) phenoxy]-	$C_{13}H_{22}OSi$	222.4	

that FTIR spectroscopy is a reliable and sensitive method for determining the composition of biomolecules [34]. The ATR-FTIR spectrum suggested the presence of various alkaloids, flavonoids, phenolics, and terpenoids, consistent with the known phytochemical profile of *S. nux-vomica* [35]. The broad and intense absorption band centered around 3375 cm^{-1} is likely attributable to the O-H stretching vibrations of hydroxyl groups, such as those found in alcohols, phenols, or carboxylic acids [28]. The peaks observed at 2925.63 cm^{-1} correspond to the C-H stretching vibrations of alkyl groups, possibly from fatty acids or terpenoids, and alkaloids [14]. The peak at 2361.21 cm^{-1} could be linked to C≡N stretching in nitrile groups, which are sometimes found in alkaloids, but sometimes due to environmental interference during sample analysis, CO_2 asymmetric stretching may arise; however, C≡N stretching is strongly supported by another peak at 1029.52 (C-N amine stretch). The peak at 1710.59 cm^{-1} can be assigned to the C=O stretching vibration of carbonyl groups, which is likely due to flavonoids, terpenoids, or esters. The absorption band around 1600 cm^{-1} may be attributed to the C=C stretching vibrations of alkene or aromatic functional groups. The peak in 1607.42 cm^{-1} represents C=C stretching in aromatic rings, characteristic of phenolic compounds or flavonoids, whereas the peak at 1452.60 cm^{-1} corresponds to C-H bending and may be indicative of the presence of the phytochemical class of terpenoids and alkanes. The peaks in 1377.41 cm^{-1} and 1073.04 cm^{-1} suggest C-O (phenol and ester stretch) and C-O (alcohols and ethers) stretching vibrations of alcohols, ethers, or esters, indicative of carboxylic acids or glycosides and flavonoids. The peak at 1266.64 cm^{-1} suggests C-O-C (ether stretch), indicative of alkaloids or glycosides [14,28].

The broad and intense absorption bands in 1073.04 cm^{-1} and 1029.52 cm^{-1} are assigned to C-O and C-N stretching, possibly from alkaloids such as strychnine and brucine. Finally, the absorption band in 669.71 cm^{-1} confirms the presence of C-H bending in aromatic compounds. Overall, the existence of flavonoids and phenolic chemicals is confirmed by the strong O-H and C=O stretching bands, which contribute to the plant's antioxidant and anti-inflammatory properties [36,37]. In addition, the C-N and aromatic stretching vibrations of C=C suggest the occurrence of strychnine and brucine, the primary bioactive alkaloids responsible for the plant's neurostimulant and analgesic effects [38]. These findings validate the plant's traditional medicinal uses and highlight the need for further GC-MS studies to quantify the identified bioactive compounds.

The GC-MS provides precise compound identification based on mass fragmentation patterns. The compound 2-butenethioic acid, S-[2-(acetylamino)ethyl] ester (RT: 9.324 min, 14.7%) contains sulfur, which may contribute to antimicrobial and anti-inflammatory properties [39]. While propane, a minor peak (RT: 9.485 min, 14.51%) itself is a simple hydrocarbon, its potential origins may be as a residual solvent. Its presence might not be biologically significant. The compound cis-aconitic anhydride (RT: 9.778 min, 11.56%) is a derivative of aconitic acid. This compound plays a role in the citric acid cycle and is known for its potential in energy metabolism and bioactive applications [40]. Notable compound cystamine (RT: 10.029 min, 10.96%) is a sulfur-containing compound studied for its antioxidant and neuroprotective effects [41]. Next, 3-Chloro-N-[2-methyl-4(3H)-oxo-3-quinazolinyl]-2-thianaphthenecarboxamide (RT: 10.134 min, 9.5%) and this compound belongs to the quinazolinone derivatives, which are known for their antimicrobial and anticancer activities [42]. Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl)- (RT: 10.217 min, 9.44%) is a carboxamide derivative that may contribute to bioactivity, though its specific pharmacological role requires further study. The compound tridecanoic acid, methyl ester (RT: 27.96 min, 2.96%) is a fatty acid methyl ester, often associated with antimicrobial and anti-inflammatory properties. Finally, silane, trimethyl [5-methyl-2-(1-methylethyl)phenoxy]- (RT: 47.251 min, 4.91%) is an organosilicon compound that has industrial applications, but some studies suggest it may have unique biological activities [43].

CONCLUSION

In this study, the UV-Vis spectrum confirmed the presence of phenolics, alkaloids, and sulfur-based compounds. The FTIR spectrum validates

functional groups such as C=O, N-H, C=C, S-S, and the GC-MS data provide specific compound identities with potential antioxidant, antimicrobial, and medicinal applications. The presence of bioactive molecules, including phenolics, carboxamides, and quinazolinones, supports the traditional medicinal use of *S. nux-vomica*. The high abundance of sulfur-containing and nitrogenous compounds suggests potential therapeutic applications in antioxidant, antimicrobial, and neuroprotective treatments. This analysis confirmed that *S. nux-vomica* contains multiple bioactive compounds, supporting its traditional medicinal use. However, further pharmacological and toxicological studies are needed to confirm their medicinal relevance.

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AUTHOR'S CONTRIBUTION

Ramu C: Original draft writing, data curation, experimental design, methodology, and conceptualization. Gayathri N: Project management, data curation, experimental design, conceptualization, formal analysis, and original draft writing. Priya G: Validation, software, formal analysis, data curation, and visualization. Sathish Kumar Boobalan: Conceptualization, methodology, experimentation, data curation, formal analysis, software, writing (original draft), visualization, validation, review, and editing. Sekar T: The research idea, methodology, experimental design, data curation, supervision, original draft writing, visualization, validation, review, and critical editing.

CONFLICT OF INTEREST

Regarding this article's publication, the authors state that they have no conflicts of interest.

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