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Phytochemical Analysis and Bioactive Compound Identification in *Strychnos nux-vomica* L.

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Abstract

This study employed GC-MS analysis to investigate the bioactive compounds present in *Strychnos nux-vomica* L., a plant renowned for its insecticidal and repellent properties. The research aimed to document plants with potential to develop new plant-based insecticides, repellents, and larvicides. Phytochemical screening of the ethanol extract revealed the presence of alkaloids, flavonoids, steroids, saponins, phenols, and proteins. GC-MS analysis identified 24 distinct compounds, elucidating the plant's chemical composition. Given the growing demand for eco-friendly rice pest management solutions, *Strychnos nux-vomica* L. emerges as a valuable source of beneficial phytocomponents, with potential applications in repellents and reproductive inhibitors.

***Key words*:** *Strychnos nux-vomica* L., Identification, Phytochemical screening, Ethanol extract GC-MS analysis

Plants have been used for centuries to fight off blood-sucking insects, and today, many regions still utilize plant-based products for this purpose [1]. Certain plants possess properties that deter pests, and they utilized these plants in various forms—such as oils, extracts, or simply crushed leaves—to protect themselves and their livestock. Recently, researchers have focused on plant extracts and natural chemicals as potential solutions for controlling these pests [2]. Various extracts from different plant types have been tested, showing potential as new insecticides and larvicides [3]. Repellents are particularly important in minimizing human contact with these insects, which helps prevent the spread of diseases carried by them [4]. Several studies have shown that plant extracts can repel *Sitophilus* species [5]. However, there is limited information on the effectiveness of *Strychnos nux-vomica* and *Lepidium sativum* against *Sitophilus oryzae* [6]. One study revealed that the ethyl acetate extract from dried *Illicium verum* fruits had a high repellent rate of 76.9% against *Sitophilus zeamais*, followed by extracts from petroleum ether and methyl alcohol [7]. Medicinal plants also contain many chemical compounds that can be used to create drugs with specific health benefits [8]. They are essential sources for developing new medications [9]. Phytochemicals found in leaves, fruits, seeds, bark, and roots help protect against various diseases [10].

Phytochemicals can be divided into primary and secondary metabolites [11]. Primary metabolites include substances like chlorophyll, proteins, and simple sugars. Secondary metabolites consist of terpenoids, alkaloids, flavonoids, saponins, and phenolic compounds [12]. Terpenoids have significant effects against inflammation, cancer, malaria, and various infections [13]. Alkaloids are valued for their pain relief, antimicrobial properties, and effectiveness against high blood pressure and malaria, making them vital in many medicinal plants [11].

Strychnos nux-vomica's primary bioactive effects are attributed to the alkaloid strychnine, which induces severe convulsions by stimulating both motor and sensory ganglia in the spinal cord, resulting in elevated blood pressure [14]. Another alkaloid, brucine, exhibits similar yet less potent effects, causing motor nerve paralysis and increased blood pressure [11]. In traditional Indian practice, S. nux-vomica seeds undergo a detoxification process involving soaking in water and milk, followed by boiling in milk [14]. Modern analytical techniques, such as HPLC and HPLC-UV, enable the monitoring of toxicity and quality in products derived from these seeds [11]. Additionally, *Strychnos nux-vomica* is employed in homeopathic medicine, although its efficacy for specific conditions remains unsubstantiated [14]. This study focuses on characterizing the chemical constituents present in the ethanol extract of *Strychnos nux-vomica*.

MATERIALS AND METHODS

Strychnos nux-vomica plants were collected from different sites in the Trichy district of Tamil Nadu. Their identification and authentication took place at the Department of Botany, St. Joseph's College, Tiruchirappalli. The collected plants were carefully washed and divided into leaves, buds, and stems. These parts were shade-dried at room temperature. After drying, the plant material was ground into a powder and extracted using ethanol, which was then analyzed for phytochemical content. Plants produce many chemical compounds, including alkaloids, flavonoids, steroids, saponins,

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phenols, and proteins, which have medicinal properties [15]. The prepared extracts underwent preliminary phytochemical screening through standard tests [16].

To test for alkaloids, the solution was mixed with Mayer's reagent. A creamy white precipitate indicated their presence [14]. To test for flavonoids, the solution was combined with 10% ammonia and heated. A fluorescent yellow color showed they were present [17]. For steroids, the solution was mixed with chloroform and acetic anhydride, then treated with concentrated sulfuric acid. A blue or green color confirmed their presence [18]. In testing for saponins, the solution was shaken with distilled water. The lack of foam indicated they were absent [19]. To check for phenols, a few drops of concentrated nitric acid were added to the plant extract. A yellow color confirmed their presence [20]. For proteins, the same method was used; a yellow color also indicated their presence [21].

Gas chromatography-mass spectrometry (GC-MS) analysis was performed with a Perkin-Elmer Clarus 680 system and a fused silica capillary column. Helium gas served as the carrier gas at a steady flow rate of 1 mL/min. For the GC-MS detection, electron ionization at 70 eV was used, with a scan time of 0.2 seconds and a mass range of 40–600 m/z. A 1 µL sample was injected at a split ratio of 10:1, with the injector set to 250 °C. The column oven started at 50 °C for 3 minutes, then increased by 10 °C per minute to reach 280 °C, before finally rising to 300 °C for 10 minutes [22]. Phytochemicals in the samples were identified by comparing their retention times, peak areas, peak heights, and mass spectral patterns with known compounds in the National Institute of Standards and Technology (NIST) spectral library [23].

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening of the alcoholic extracts from the leaves, seeds, and bark of *Strychnos nux-vomica* revealed the presence of several bioactive compounds, indicating the plant's potential medicinal properties.

Table 1 Phytochemical from leaves extract of *Strychnos Nux-vomica*

Phytoconstituents	Alcoholic extract
Alkaloids	+
Carobhydrates	+
Glycosides	+
Phytosterols	+
Saponins	-
Fixed oils / Fats	-
Phenols and Tannins	+
Proteins	+
Amino acids	-
Gum and Mucilage	+
Flavonoids	+
Lignin	+

(+) Presence (-) Absence

Leaves

The phytochemical analysis of the leaves of *Strychnos nux-vomica* showed the presence of various important bioactive constituents. Alkaloids, carbohydrates, glycosides, phytosterols, phenols, proteins, gum, and mucilage were detected in the leaf extract. Flavonoids and lignin were also present, which are compounds known for their antioxidant, anti-inflammatory, and other health-promoting properties. The absence of saponins, fixed oils/fats, and amino acids suggests that these constituents are not prominent in the leaves of the plant.

Table 2 Phytochemical from seeds extract of *Strychnos Nux-vomica*

Phytoconstituents	Alcoholic extract
Alkaloids	+
Carobhydrates	+
Glycosides	+
Phytosterols	+
Saponins	-
Fixed oils / Fats	-
Phenols and Tannins	+
Proteins	-
Amino acids	-
Gum and Mucilage	+
Flavonoids	+
Lignin	+

(+) Presence (-) Absence

Table 3 Phytochemical from bark extract of *Strychnos Nux-vomica*

Phytoconstituents	Alcoholic extract
Alkaloids	+
Carobhydrates	+
Glycosides	+
Phytosterols	+
Saponins	-
Fixed oils / Fats	-
Phenols and Tannins	+
Proteins	-
Amino acids	-
Gum and Mucilage	+
Flavonoids	+
Lignin	+

(+) Presence (-) Absence

Seeds

Similar to the leaves, the seed extract of *Strychnos nux-vomica* exhibited the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenols, gum, and mucilage. Flavonoids and lignin were also identified, supporting the seeds' potential medicinal value. However, the seed extract did not contain saponins, fixed oils/fats, proteins, or amino acids, which further emphasizes the specific biochemical profile of the seeds.

Bark

The phytochemical screening of the bark revealed a composition very similar to that of the leaves and seeds. Alkaloids, carbohydrates, glycosides, phytosterols, phenols, gum, and mucilage were found in the bark extract. Flavonoids and lignin were also present, which contribute to the plant's overall therapeutic potential. As with the leaves and seeds, the bark did not contain saponins, fixed oils/fats, proteins, or amino acids.

GC-MS analysis

The GC-MS analysis of the ethanolic extract of *Strychnos nux-vomica* identified 23 distinct compounds with a variety of chemical properties. Each compound was characterized based on its molecular formula, molecular weight, and retention time (RT) by comparing the mass spectral data with entries in the National Institute of Standards and Technology (NIST) library. These compounds indicate the plant's potential as a source of bioactive molecules with diverse pharmacological applications.

Table 4 Identified compounds from Leaves, Seeds and Bark extract of *Strychnos Nux-vomica*

S. No	Compound name	Molecular formula	Molecular weight (g/mol)	Retention time (RT)
1	Methyl Hydrogen Disulfide	CH_4S_2	480	-
2	Acetamide, N-Methyl-2-(Methylamino)-2-Thioxo-	$C_4H_8ON_2S$	408	-
3	Propyl Mercaptan	C_3H_8S	471	-
4	(5-Nitro-1H-1,2,4-Triazol-3-yl)acetic Acid	$C_4H_4O_4N_4$	401	-
5	Formic Acid, 1-Methylethyl Ester	$C_4H_8O_2$	443	-
6	Carbamimidoylsulfanylacetic Acid	$C_3H_6O_2N_2S$	376	-
7	(Methylthio)-Acetonitrile	C_3H_5NS	441	-
8	Thiazolidin-4-One, 5-Ethyl-2-Imino-	$C_5H_8ON_2S$	322	-
9	Propanethial, S-Oxide	C_3H_6OS	421	-
10	4-Penten-2-ol	$C_5H_{10}O$	372	-
11	S-Methylpropanethiosulfonate	$C_4H_{10}O_2S_2$	408	-
12	2-Propanol, 1-Methoxy	$C_4H_{10}O_2$	409	-
13	4-Penten-2-ol	$C_5H_{10}O$	365	-
14	2-Pyrrolidinethione	C_4H_7NS	405	-
15	Formic Acid, 1-Methylethyl Ester	$C_4H_8O_2$	355	-
16	Thiazolidin-4-One, 2-Hydroxymethylimino-	$C_4H_6O_2N_2S$	417	-
17	Paraldehyde	$C_6H_{12}O_3$	302	-
18	Methylene Diamine, N,N'-Diacetyl-	$C_5H_{10}O_2N_2$	339	-
19	Carbamimidothioic Acid, Methyl Ester	$C_2H_6N_2S$	374	-
20	Methyl Thiolacetate	C_3H_6OS	315	-
21	S-Methylpropanethiosulfonate	$C_4H_{10}O_2S_2$	358	-
22	Propanoic Acid, 3-(Acetylthio)-2-Methyl-	$C_6H_{10}O_3S$	331	-
23	Ethyl N-Hydroxyacetimidate	$C_4H_9O_2N$	342	-

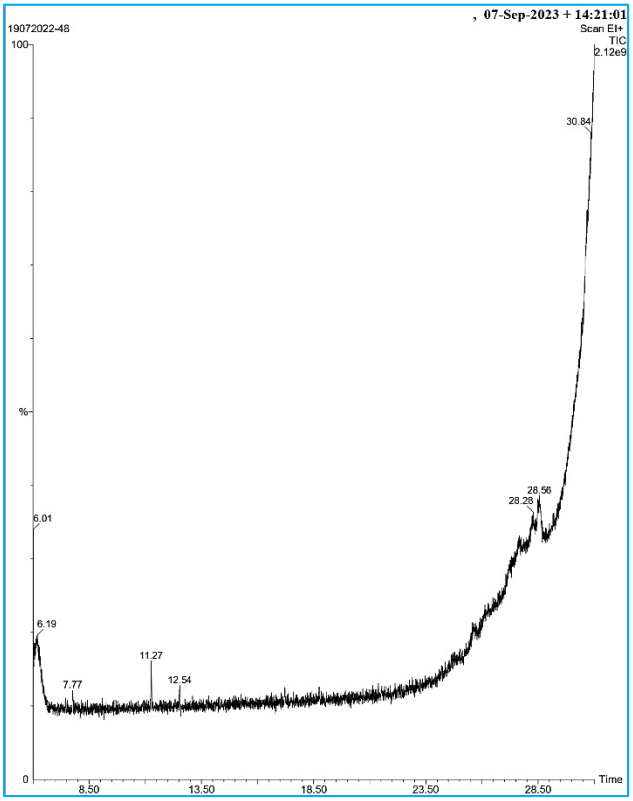

Fig 1 GC-MS Chromatogram of ethanol extract of *Strychnos Nux-vomica* leaves

Identified compounds

The analysis revealed the presence of sulfur- and nitrogen-containing compounds such as Methyl Hydrogen Disulfide (CH_4S_2; M. Wt: 480) and Acetamide, N-Methyl-2-(Methylamino)-2-Thioxo- ($C_4H_8ON_2S$; M. Wt: 408). These compounds are known for their antimicrobial and antioxidant properties. Similarly, Propyl Mercaptan (C_3H_8S; M. Wt: 471) and Thiazolidin-4-One, 5-Ethyl-2-Imino- ($C_5H_8ON_2S$; M. Wt: 322) are recognized for their antimicrobial and anti-inflammatory activities, respectively. The presence of these compounds highlights the therapeutic potential of *Strychnos nux-vomica*.

Other notable compounds include Formic Acid, 1-Methylethyl Ester ($C_4H_8O_2$; M. Wt: 443) and 4-Penten-2-Ol ($C_5H_{10}O$; M. Wt: 372), which are associated with antioxidant properties. The detection of S-Methylpropanethiosulfonate ($C_4H_{10}O_2S_2$; M. Wt: 408) and Paraldehyde ($C_6H_{12}O_3$; M. Wt: 302) adds to the extract's pharmacological significance, as these compounds exhibit antibacterial and sedative activities, respectively.

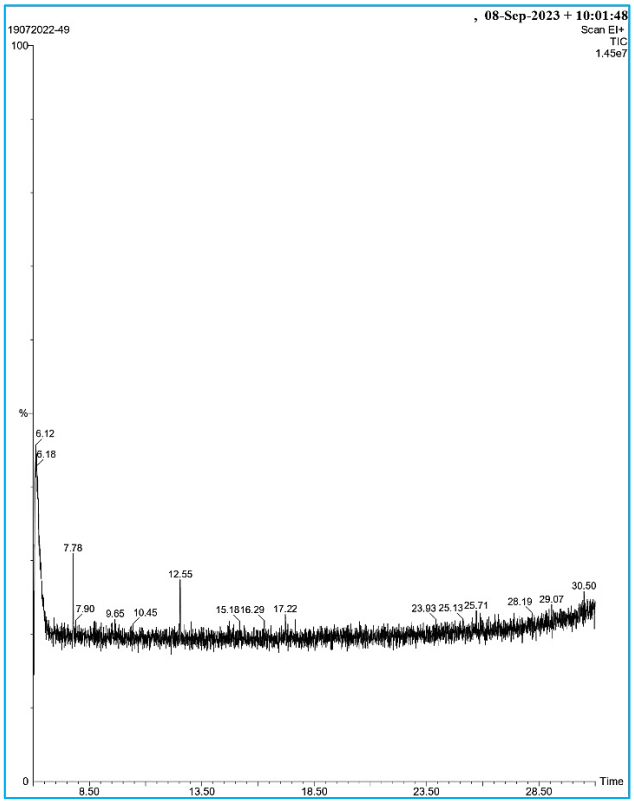

Fig 2 GC-MS Chromatogram of ethanol extract of *Strychnos Nux-vomica* seeds

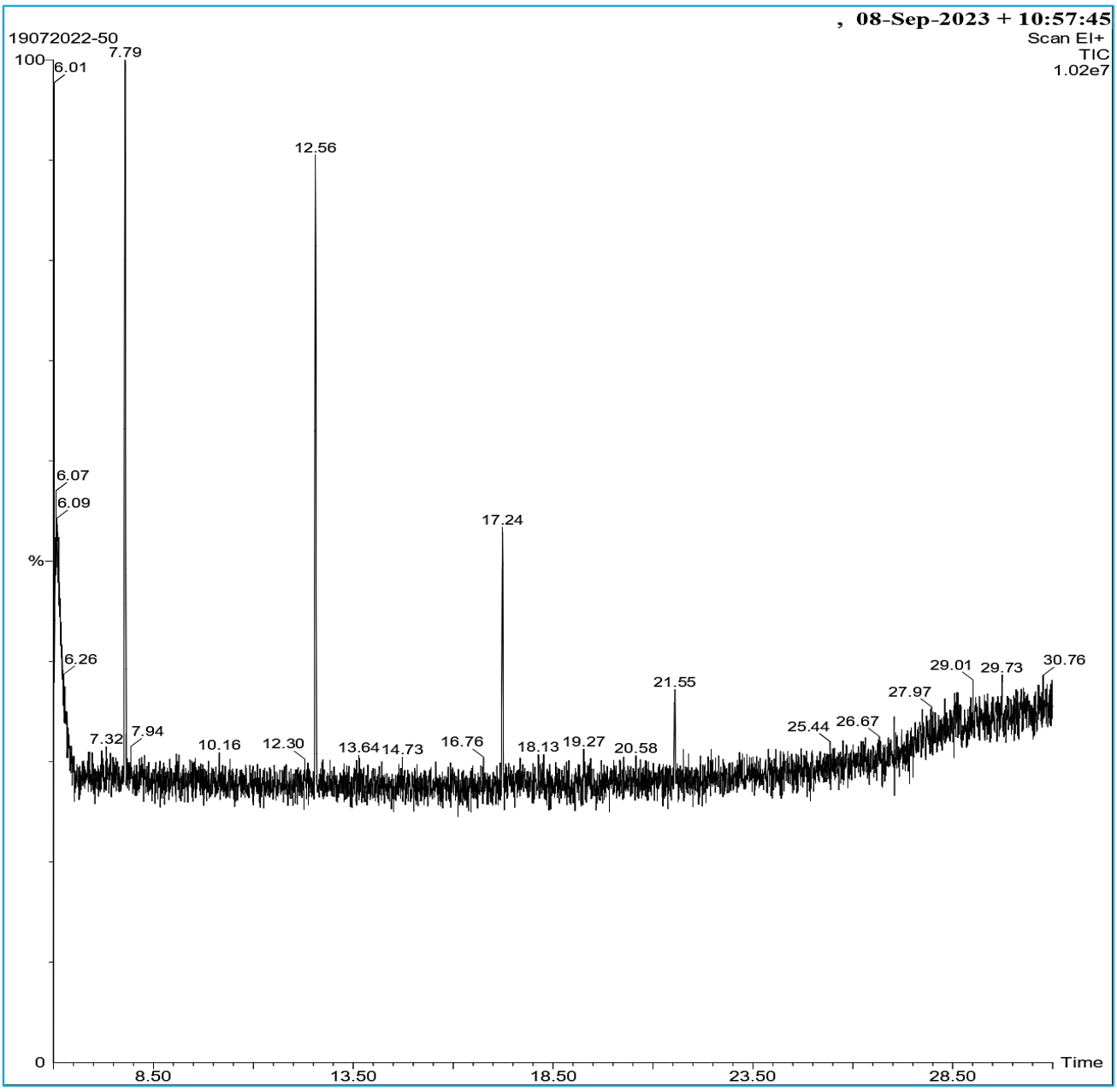

Fig 3 GC-MS Chromatogram of ethanol extract of *Strychnos Nux-vomica* bark

Phytochemical screening correlation

Preliminary phytochemical screening of the ethanolic extract confirmed the presence of alkaloids, flavonoids, phenols, and proteins. These findings align with the GC-MS results, as several identified compounds contain functional groups associated with these phytochemicals. For instance, nitrogen-containing compounds such as Acetamide derivatives and Thiazolidinones correspond to alkaloids, while alcohol-based compounds like 4-Penten-2-Ol are consistent with flavonoids. Similarly, phenolic compounds were indirectly detected through ester and acid molecules such as Formic Acid derivatives.

Unique and rare compounds

Some compounds identified in this study, such as Carbamimido sulfanylacetic acid ($C_3H_6O_2N_2S$) and (Methylthio)-Acetonitrile (C_3H_5NS), are rarely reported in *Strychnos* species. These findings suggest potential novel bioactivities and warrant further exploration of their therapeutic applications. The diversity of compounds detected reflects the rich phytochemical profile of *Strychnos nux-vomica*, which is known for its traditional use in medicine.

Chemical distribution

The retention time and molecular weight data indicate that the extract contains both low- and high-molecular-weight compounds, each contributing uniquely to the plant's pharmacological potential. Low-molecular-weight compounds, such as Methyl Hydrogen Disulfide, are associated with quick pharmacological action, while higher molecular-weight compounds, such as Carbamimido Thioic Acid, Methyl Ester, are typically linked to prolonged biological activity [24].

CONCLUSION

Phytochemical screening of *Strychnos nux-vomica* reveals a diverse array of bioactive compounds in its leaves, seeds, and bark, including alkaloids, flavonoids, glycosides, phenols, and lignin. These compounds are associated with various medicinal benefits, while the presence of phytosterols and tannins further underscores the plant's therapeutic potential. Conversely, the absence of saponins, fixed oils, fats, proteins, and amino acids suggests these compounds are not major constituents of the plant. These findings provide a solid foundation for further pharmacological investigations to unlock

the full medicinal value of *Strychnos nux-vomica*. GC-MS analysis of the ethanolic extract of *Strychnos nux-vomica* provides a comprehensive overview of its complex chemical composition. The identified bioactive compounds validate the plant's traditional medicinal uses and highlight its potential for developing novel therapeutic agents. Future studies, including biological activity assays, are recommended to validate these findings and explore the full spectrum of pharmacological benefits offered by the identified compounds.

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