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GC-MS Analysis of volatile components extracted from *Strychnus nux-vomica* Linn. seeds

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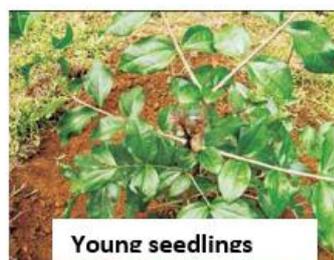
Abstract

Medicinal plants have had a crucial role in human culture as well as in agriculture, as bio-pesticides. The plant, *Strychnus nux-vomica* is a medicinal plant and traditionally seeds of *S. nux-vomica* are used for therapeutic ailments. A Gas Chromatography Mass-Spectroscopy analysis was carried out to identify the biochemical components present in the seeds of *S. nux-vomica* seeds. A wide range of compounds, including alkaloids such as strychnine, brucine, vomicine, isostrychnine, pseudostrychnine, chlorogenic acid etc were identified in the hexane extracts of the seeds, which is found to be responsible for insecticidal activity against insect pests. Hence, it can be recommended as a bio-pesticide having anti-insecticidal activity for controlling agricultural pests.

Keywords: GC-MS, *Strychnus nux-vomica*, Phytochemical compounds, medicinal plants

Introduction

The strychnine tree (*Strychnos-nux-vomica*) commonly known as poison nut, snake-wood, semen strychnos or Quaker buttons, is a highly poisonous medium-sized deciduous tree, which belongs to the family loganiaceae that grows in open habitats. The strychnine tree (*Strychnos nux-vomica* L.), also known as strychnine tree, is a deciduous tree native to India, and southeast Asia. *Strychnus nux-vomica* belongs to the family Strychnaceae (Loganiaceae), commonly known as nux-vomica, poison nut, snake wood, strychnine tree, quaker buttons, semen strychnos in english, yetti in Tamil and kanjiram in Malayalam. The species is indigenous to India and is distributed in moist deciduous forests throughout the tropical India. Cultivation of *S. nux-vomica* extends from Sri Lanka, Southern China, South East Asia and Northern Australia. *S. nux-vomica* species is a medium sized, deciduous tree, with fairly straight and cylindrical bole and dark-grey or yellowish-grey bark with minute tubercles. The *nux-vomica* grows as tall as 49.2 feet (15 m) and has round, opposite leaves and attractive white flowers. Leaves are simple, opposite, orbicular to ovate, 6 to 12 cm broad, coriaceous, glabrous and five nerved. Flowers are white and fragrant. Flowering occurs from March to May and fruits mature up to December. Spherical fruits of the *nux-vomica* are large and hard-rinded. Berries contain 3 to 8 round, flattened, greyish seeds. Seeds are covered with silky hairs, hard and extremely bitter in taste. *Strychnos nox-vomica* seeds contain a mixture of 13 alkaloids (Yang and Yan, 1993) [4] but the main alkaloids are strychnine and brucine (Han, 1988). Content of strychnine and brucine may vary in different plant parts. Seeds of *S. nux-vomica* contain 0.4 and 0.6% strychnine and brucine, respectively. Traditionally seeds of *S. nux-vomica* are used for therapeutic ailments. It has antitumor, antimicrobial, anti convulsion, anti amnesic and immunomodulatory effects (Arun kumar *et al.*, 2012). Insecticidal activity of *S. nux-vomica* extracts has been reported against mosquitoes and beetles (Chandel *et al.*, 1987; Arivoli, 2012) [3, 2].



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Plate 1: Description of *Strychnos nux-vomica*

Other parts of the tree have varying percentage of these two alkaloids viz., 1.7 and 2.8% in root bark, 0.3 and 0.4% in root-wood, 0.9 and 2.1% in stem bark, 0.5 and 0.01% in stem wood and 0.2 and 0.5% in leaves, respectively. Keeping above in back of mind and to exploit the potential of *S. nux-vomica* as a biopesticide, a GC-MS Analysis was conducted to identify the biochemical components present in the seeds of *S.nux-vomica*.

Materials and Methods

Collection and processing of plant samples: Different plant parts viz., fresh leaves, seeds, stem bark, root bark and fruit rind of *S. nux-vomica*, each weighing almost 3 kg were collected from the trees found in drought prone area of Krishnagiri district of Tamil Nadu. The plant samples were air dried in the Entomology laboratory, TNAU, Coimbatore up to two weeks and ground into uniform powder and packed in 3 kg plastic containers separately.

Extraction of active principles from the plant samples

Dry powders of seed samples were packed in filter paper made 20cm×4.5 cm size cylindrical thimbles. The samples filled thimbles were kept in the cylindrical sample holder present in the soxhelt apparatus and filled with organic solvent, hexane. Plant samples were extracted with organic solvents. When hexane was mixed with seed sample it produced coloured solution, extraction was done until the coloured solvent became transparent. During extraction process temperature maintenance was an essential task as over temperature leads to explosion. Temperature ranges for hexane is 69 °C. This extraction process takes approximately 12-18 h for each sample. The extracts were collected in 50 ml screw capped vials and excess solvent was evaporated in hot water bath (65 °C) and concentrated extracts were stored at 4 °C for further usage.

Table 1: Physical characteristics of *Strychnos nux-vomica* Linn. plant samples.

Name of the plant sample	Plant Parts used	Shape of samples	Colour of the samples
<i>S. nux-vomica</i>	Leaves	Orbicular to ovate	Dark green
<i>S. nux-vomica</i>	Root bark	***	Light red
<i>S. nux-vomica</i>	Stem bark	***	Dirty white
<i>S. nux-vomica</i>	Seed	Round coin like, concave one side, convex other side	Dirty white, black
<i>S. nux-vomica</i>	Fruit rind	***	Tender fruits green, mature fruits yellowish red

Characterization of hexane extracts of *S. nux-vomica* seed:

Analytical studies using Gas Chromatography Mass Spectroscopy (GC-MS) were carried out to identify the active principles present in the hexane extracts of *S. nux-vomica* seed. The results of analysis revealed that more than hundred compounds are found in the hexane extracts of *S. nux-vomica* seed. These include squalene, floridanine, strychnine, beta-

Chemical analysis of active principles from plant samples

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald, 1997) [1].

GC / MS Analysis of hexane extracts of *S. nux-vomica* seed was determined by GC - MS. Equipment: Thermo GC-Trace ultra ver: 5.0, Thermo MS DSQ II Mass Spectrometer under the following conditions: Column – DB 5 – MS Capillary Standard Non – Polar column, Dimension: 30 mts, ID: 0.25 mm, Film: 0.25 µm, Carrier gas : Helium (He), Flow at the rate of 1.0 mL/Min, Column temperature programmed at 70 °C with 6 °C/min raised to 260 °C. 1 µL of hexane extracts of *S. nux-vomica* used for injection. For GC/MS detection an ionization energy of 70ev was used.

One gram of hexane extracts of *S. nux-vomica* seed sample was taken separately and made up to 50 ml with methanol, from which 1µl of sample was injected (split mode) in the column. The components were identified based on NIST Library / Wiley Library.

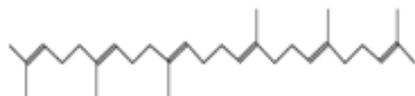
Results and discussion

The physical characters of *Strychnos nux-vomica* Linn. plant samples were described in Table 1. The leaves were orbicular to ovate in shape and dark green in colour. The root and stem barks were light red and dirty white in colour respectively. The seeds are round coin like, concave one side and convex other side and dirty white or blackish in colour. The fruit rind of tender fruits is green and mature fruits are yellowish red in colour.

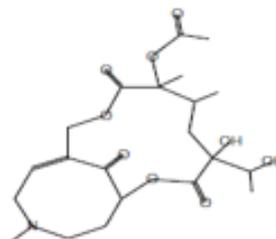
colubrine, vomicine, brucine, pseudostrychnine, chlorogenic acid, pentanoic acid, desulphosinigrin, octadeconoic acid, hexadeconoic acid, ergotamine, cis-vaccenic acid, pentadeconic acid, tetradeconic acid, etc. List of compounds fractionated and their structure, chemical formula and molecular mass were presented in Table 2.

Table 2: Chemical characterizations of hexane extracts of *S. nux-vomica* seeds.

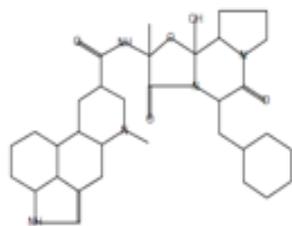
S.N	Chemical name	Molecular formula	Molecular weight	Area (%)
1.	Squalene	C ₃₀ H ₅₀	410	0.48
2.	Floridanine	C ₂₁ H ₃₁ NO ₉	441	0.11
3.	Brucine,10,11-Methoxy strychnine	C ₂₃ H ₂₆ N ₂ O ₄	394	0.16
4.	Icajine,19-methyl-16,19-secostrychnidine	C ₂₂ H ₂₇ N ₂ O ₂	144	0.16
5.	2-Propyl-tetrahydropyran-3-ol	C ₈ H ₁₆ O ₂	164	0.16
6.	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	279	1.20
7.	Xanthosine (CAS)	C ₁₀ H ₁₂ N ₄ O ₆	284	2.57
8.	Ergotamine - GC Artefact	C ₁₄ H ₁₆ N ₂ O ₂	244	0.32
9.	Guanosine (CAS)	C ₁₀ H ₁₃ N ₅ O ₅	283	2.57
10.	Strychnine, strychnidine-10-one	C ₂₁ H ₂₂ N ₂ O ₂	334	0.16
	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282	0.22
11.	2-n-Propylthiane	C ₈ H ₁₆ S	148	0.16
	Pentadecanoic acid (CAS)	C ₁₅ H ₃₀ O ₂	242	0.87
12.	Chlorogenicacid, 4,5-trihydroxycyclohexane carboxylic acid	C ₁₆ H ₁₈ O ₉	354	0.16
13.	Pentanoic acid, 2-acetyl-4-methyl-, ethyl ester	C ₁₀ H ₁₈ O ₃	186	0.16
14.	Beta-colubrine, 2-Methoxy strychnine	C ₂₃ H ₂₆ N ₂ O ₂	364	0.16
15.	Dodecanoic acid (CAS)	C ₁₂ H ₂₄ O ₂	200	2.29
16.	Quinic acid	C ₇ H ₁₂ O ₆	192	3.95
17.	(1S,10S,22R,23R,24S)Vomicine,15-hydroxy-4-methyl - pseudostrychnine	C ₂₂ H ₂₅ N ₂ O ₄	380	0.16

Chemical structures identified from *S. nux-vomica* seeds

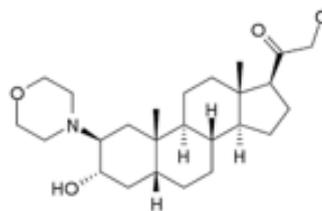
Squalene



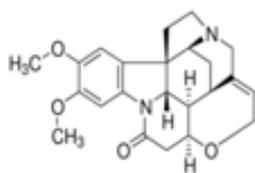
Squalene



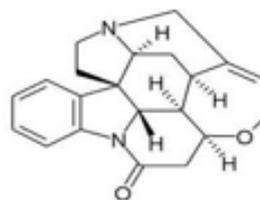
Floridanine



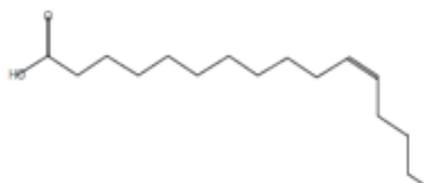
Ergotamine



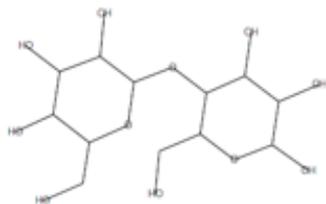
Brucine



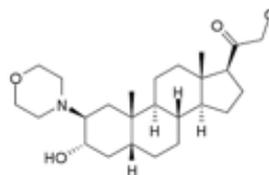
Strychnine



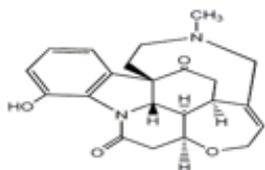
Cis-Vaccenic acid



Desulphosinigrin



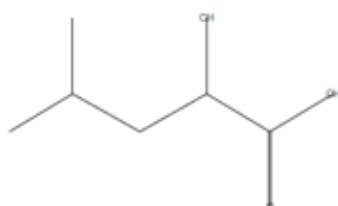
Beta-colubrine



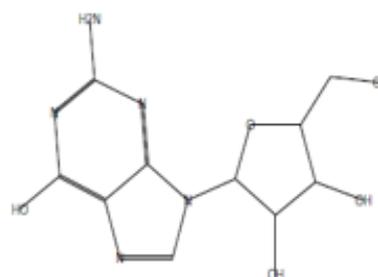
Vomisine



Octanoic acid



Pentanoic acid



Guanidine

Cai *et al.* (1990) [8] also reported that alkaloids such as strychnine, brucine, isostrychnine, isobrucine, strychnine *N*-oxide, and brucine *N*-oxide in *S. nux-vomica* L. seed through HPLC-ESI/MS method.

The main alkaloids present in the seeds of *S. nux-vomica* L. were known to be strychnine, brucine, and vomisine (Han, 1988). Sivaraman *et al.* (2014) [6] found out the presence of secondary metabolites namely steroids, terpenoids, phenols, tannins, coumarins, flavonoids, quinines, alkaloids and saponins in *S. nux-vomica* L. and *S. anacardium* L. These seed extracts were responsible for larvicidal, pupicidal, antifeedant, adult deformation activities of *Helicoverpa armigera* Hubner. According to Arivoli *et al.* (2011) [7] phytochemical constituents such as strychnine, brucine, strychnicine in *S. nux-vomica* L. were responsible for larvicidal effect to *Culex quinquefasciatus* Say.

Conclusion

Based on the information available, it is concluded that alkaloids such as strychnine, brucine, vomisine, isostrychnine, pseudostrychnine, chlorogenic acid etc., present in the *S. nux-vomica* L. This can be utilized for phyto-insecticide research.

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