International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2020; 8(1): 1112-1116

Received: 07-11-2019
Accepted: 09-12-2019

Suganthy M
Forest College and Research Institute Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Gajendra CV
Forest College and Research Institute Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Corresponding Author:
Suganthy M
Forest College and Research Institute Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Chemical characterization of *Strychnos nux-vomica* L. leaves for biopesticidal properties using GC-MS

Suganthy M and Gajendra CV

DOI: https://doi.org/10.22271/chemi.2020.v8.i1o.8398

Abstract
Strychnos nux-vomica commonly known as poison nut tree found to possess various pharmaceutical properties. The GC-MS analysis of methanolic and ethanolic leaf extracts revealed 44 compounds. Among them four compounds *viz.,* benzene, 1-2, dichloro (4.38 per cent), dibutyl phthalate (5.20), hexadecanoic acid, ethyl ester (0.91per cent) and pentadecane (1.24 per cent) posses biopesticidal property. Hence, there is a scope of utilizing *Strychnos nux-vomica* as botanical pesticide for eco-friendly management of insect pests.

Keywords: *Strychnos nux-vomica,* chemical profiling, GC-MS, biopesticide

Introduction
Strychnos nux-vomica L. belongs to the family Loganiaceae and is commonly known as poison nut tree, venom orange, quaker buttons. It is a deciduous tree native to India and to South-East Asia. Strychnos trees are the best source of highly poisonous alkaloids *i.e.* strychnine, brucine and loganine (Gupta *et al*., 2014) [4]. Insect pests, diseases and weeds are important biotic constraints inflicting 25 to 30 per cent loss in agriculture production. Synthetic pesticides have of course, played very significant role in restricting many pest problems. However, indiscriminate use of chemicals has resulted in pesticide resistance, resurgence of target organism or emergence of secondary pests because of destruction of parasitoids and predators, impact on non-target organisms, including humans, environmental pollution through accumulation of pesticides in soil, water and air and residues in agricultural and animal products. Increasing awareness about the deleterious effects of insecticides paved the way for eco-friendly pest management. Biopesticides offer numerous advantages over traditional, chemical or synthesized pesticides. They are often safer to the non-target organisms compared to conventional pesticides and also the biopesticides can offer much more targeted activity against a desired pest, as opposed to conventional pesticides, which often affect a broad spectrum of pests along with birds and mammalian species. Biopesticides often are effective in very small quantities there by offering lower exposure (Thakore, 2006) [14].

In recent years there is a vast demand to find an alternative to chemical pesticides. Interest on manufacturing of botanical or plant based insecticides including essential oil and plant extracts is increasing due to environmental concern and problems experienced with development of resistance in insects to conventional chemical insecticides (Hamada *et al.,* 2018). Plant based insecticides are gaining momentum, especially in the field of organic agriculture due to the problems of chemical pesticide *viz*., residues in the crop produce, development of insecticide resistance in pests and resurgence of minor pests attaining the status of major pests.

The present investigation aims at characterizing the bioactive compounds in methanolic and ethanolic extracts of *S. nux-vomica* leaves using Gas Chromatography and Mass Spectrometry (GC-MS). Currently the Gas Chromatography and Mass Spectrometric methods play vital role in screening and identification of various secondary metabolite profiling and helps to evaluate the biopesticidal and pharmaceutical properties of bioactive compounds.

Materials and Methods
Collection and processing of leaf sample
Leaves of *S. nux-vomica* were collected from trees of Forest College and Research Institute, Mettupalayam campus of Tamil Nadu Agricultural University, Coimbatore.

The collected leaf samples were washed thoroughly with tap water and shade dried at room temperature (25±2 ºC) for a period of 10 to 15 days until the leaves were completely dried. The dried samples were powdered using mixer grinder, sieved to a fine powder and stored in air tight glass containers for further analysis.

Preparation of crude extract

Exactly five grams of powdered leaf sample of *S. nux-vomica* was packed in a filter paper and made into 20 cm × 4.5 cm size cylindrical thimbles. The sample filled thimbles were kept in the cylindrical sample holder present in the soxhlet apparatus and filled with organic solvents such as ethanol and methanol, individually. Plant samples were extracted with the said organic solvents. Organic solvents when mixed with plant sample produced coloured solution. Extraction was continued till the coloured solvent became transparent. During the extraction process, temperature of soxhlet apparatus set up was maintained at 79 ºC for ethanol and 65 ºC for methanol extracts. The extraction process has taken approximately 6 to 8 hours for each sample. Both the extracts were concentrated under room temperature and dried crude extracts were subjected to further analysis.

Gas Chromatograph and Mass Spectrometry (GC-MS) analysis

The phytochemical composition of ethanolic and methanolic extracts of *S. nux-vomica* leaves was analysed using Shimadzu Gas Chromatography and Mass Spectrometry (GC-MS) - QP 2020 with a SH-Rxi-5 Sil MS Cross Band (similar to 5% diphenyl 95% dimethyl polysiloxane) capillary mid-polar column (30 m, ID: 0.25 mm and film thickness of 0.25 µm). Sample sizes of 1µl of each ethanolic and methanolic extracts were injected separately for analysis and Helium was used as a carrier gas at 1.2 ml/minute. The oven temperature was programmed from 80 ºC to 285 ºC (80 ºC for 5 min, 4 ºC rate 260 ºC, and 2 ºC rate 285 ºC hold for 10 minutes). The MS was set to scan from 45 to 650 Da. The MS also had inbuilt pre-filter which reduces the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum *viz.,* NIST4 and WILEY9 containing more than million references. The interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 libraries. The relative percentage of extract constituent was expressed with peak area normalization.

Results and Discussion

In ethanolic extract, 25 compounds were identified. In terms of peak area propane, 1,1,3-triethoxy constitutes 26.79 per cent followed by 2,4-pyrrolidinedione (9.04 per cent), heptane, 2,4-dimethyl (7.79 per cent), tetradecane (6.80 per cent), phytol (5.24 per cent), dibutyl phthalate (5.21 per cent) and the least was 1-hexadecanol (0.30 per cent) with the retention time of 9.906, 5.992, 3.159, 15.579, 23.945, 22.389 and 15.46 minutes, respectively (Table 1 and Figure 1).

The major compounds in methanolic extract were mome inositol (23.51 per cent), dibutyl phthalate (11.00 per cent), n-pentadecanol (8.81 per cent), 1-methylcyclohexyl acetate (8.04 per cent), (4E)-4-[(E)-but-2-enylidene]-3,5,5-trimethyl-1-cyclohex-2-enone (6.68 per cent) and the least was pentadecane (1.24 per cent) with retention time 15.40, 17.759, 14.873, 15.445, 15.267 and 13.096 minutes, respectively as indicated in Table 2 and Figure 2. Among these compounds, benzene, 1-2, dichloro, dibutyl phthalate, hexadecanoic acid, ethyl ester and pentadecane were found to have biopesticidal activity and structures are elucidated in Figure 3

The results are in-line with earlier findings of various leaf extracts in methanolic and ethanolic solvents. Parasuraman *et al.* (2009) identified 17 compounds in methanolic extract of *Cleistanthus collinus*. Ravishanker and Ester (2017) reported 21 compounds in methanolic extract of *Hydrophila auriculata*. Sivaraman *et al*. (2014) [13] found out the presence of secondary metabolites namely steroids, terpenoids, phenols, tannins, coumarins, flavonoids, quinines, alkaloids and saponins in *Strychnus nux-vomica* and *Semicarpus anacardium*. These plant extracts were found to be responsible for larvicidal, pupicidal, antifeedant, adult deformation activities of *Helicoverpa armigera*. According to Arivoli *et al*. (2011) phytochemical constituents such as strychnine, brucine, strychnicine in *S. nux-vomica* were responsible for larvicidal effect to *Culex quinquefasciatus*. Selvaraj *et al*. (2018) [11] found 17 compounds in hexane extracts of *S. nux-vomica* seed and the major are xanthosine, dodecanoic acid, quinic acid, guanosine and desulphosinigrin. Tyagi and Agarwal (2017) [15] reported several compounds in methanolic extract of *Pistia statoides* (8) and *Eichhornia crassipes* (12). Madkour *et al*. (2017) [6] extracted 27 compounds using methylene chloride and 46 compounds using n-hexane from the leaves of *Senna italica*. Elaiyaraja and Chandramohan (2016) [2] reported that *Indoneesiella echioides* yielded 9 compounds in ethanol, 10 in butanol, 7 in methanol, 5 in acetone, 9 in chloroform and 13 compounds in n-hexane solvent. Uraku (2016) [16] extracted *Spilanthes uliginosa* and reported 6 compounds. Amudha and Rani (2014) recorded 20 compounds in ethanolic extract of *Cadaba fructicosa*. Muthulakshmi *et al*. (2012) [7] reported 18 compounds in *Ferronia elephantum*. Sermakkani and Thangapani (2012) recorded 17 compounds in *Cassia italica*. Gopalakrishnan and Udayakumar (2014) documented 39 compounds from *Marsilea quadrifolia*. Rahman *et al*. (2014) [9] reported that methanolic extracts of *Jatropha curcas*, *Psidium gujava* and *Andrographis paniculata* yielded 9, 12 and 29 compounds, respectively.

Table 1: List of bioactive compounds present in ethanolic extract

S. No.	Retention time (min)	Compound name	Peak area	Biological activity
1	3.159	Heptane, 2,4-dimethyl-	7.79	Antimicrobial
2	3.702	Heptanoic acid	1.15	Flavouring agent
3	4.304	Propane, 1,1-diethoxy-2-methyl-	2.00	Flavouring agent
4	5.810	2-Butene, 1-chloro-4-ethoxy-	0.39	Antibacterial
5	5.992	2,4-Pyrrolidinedione	9.04	Antibacterial, Antifungal
6	6.996	Butane, 1,1-diethoxy-3-methyl-	1.42	Anti-inflammatory
7	7.205	3,3-diethoxy-2-butanone	0.83	-
8	8.252	Pentadecane	2.44	Antibacterial
9	8.550	Octyl 3-hydroxypropanoate	0.56	-
10	8.591	Benzene, 1,2-dichloro-	4.38	Fumigants, Insecticides

11	9.906	Propane, 1,1,3-triethoxy-	26.79	Antibacterial
12	10.450	5-Trimethylsilylhex-3-yn-5-en-1-ol	0.81	-
13	12.194	Azulene	3.83	Anti-inflammatory
14	12.407	Dodecane	4.85	Antibacterial
15	15.460	1-Hexadecanol	0.30	Cosmetics
16	15.579	Tetradecane	6.80	Antimicrobial
17	18.276	Nonadecane	4.64	Anti-fungal and antimicrobial
18	18.354	1,3,4,5-Tetrahydroxy cyclohexane carboxylic acid	3.70	-
19	18.679	(4Z)-4-[(Z)-but-2-enylidene]-3,5,5-trimethyl-1-cyclohex-2-enone	1.87	-
20	20.671	Tricosane	2.38	Biopesticide
21	21.745	4,6-Dimethyl-3-nonanone	1.61	Photo anti-proliferative
22	22.389	Dibutyl phthalate	5.20	Ecto-parasiticide
23	22.740	Hexadecanoic acid, ethyl ester	0.91	Antifungal, Nematicidal, Pesticidal
24	22.832	6-(3,4-Dichlorophenyl)-4,5-dimethyl-8,9-dihydrofuro[2,3-h]chromen-2-one	1.09	-
25	23.945	Phytol	5.24	Antimicrobial

Table 2: List of bioactive compounds present in methanolic extract

S. No.	Retention time (min.)	Compound name	Peak area	Biological activity
1	2.635	2,2-Dimethoxybutane	1.76	Antimicrobial, antibacterial
2	10.785	Piperidine, 4-methyl-1-nitroso-	2.35	Antimicrobial
3	11.423	Cyclohexane, 1,1'-(1,5-pentanediyl)bis-	1.42	Antibacterial
4	12.804	(E)-2,3-Epoxy-1-(methoxy methoxy) tetradecane	1.52	Antibacterial
5	13.014	1-Tetradecanol	4.85	Cosmetics, Insect attractant
6	13.096	Pentadecane	1.24	Household pesticides
7	14.873	n-Pentadecanol	8.81	Antibacterial, antimicrobial
8	15.040	1,3,4,5-Tetrahydroxy cyclohexane carboxylic acid	6.63	-
9	15.195	Octadecanoic acid	1.38	Antimicrobial
10	15.267	(4E)-4-[(E)-but-2-enylidene]-3,5,5-trimethyl-1-cyclohex-2-enone	6.68	-
11	15.400	Mome inositol	23.51	Antioxidant
12	15.445	1-Methyl cyclohexyl acetate	8.04	Antibacterial
13	15.505	Cyclohexane, hexyl-	3.31	Antibacterial
14	15.546	4-Undecanone	1.93	Antimicrobial
15	16.514	1-Docosanol	6.43	Antiviral
16	16.850	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	2.77	-
17	17.136	10-Nonadecanone	1.63	Antimicrobial, Antioxidant
18	17.759	Dibutyl phthalate	11.00	Ectoparasiticide
19	17.991	1-Hexacosanol	3.09	Antibacterial
20	18.801	4-Isopropyl-1-methylcyclohexanol	1.65	Antibacterial

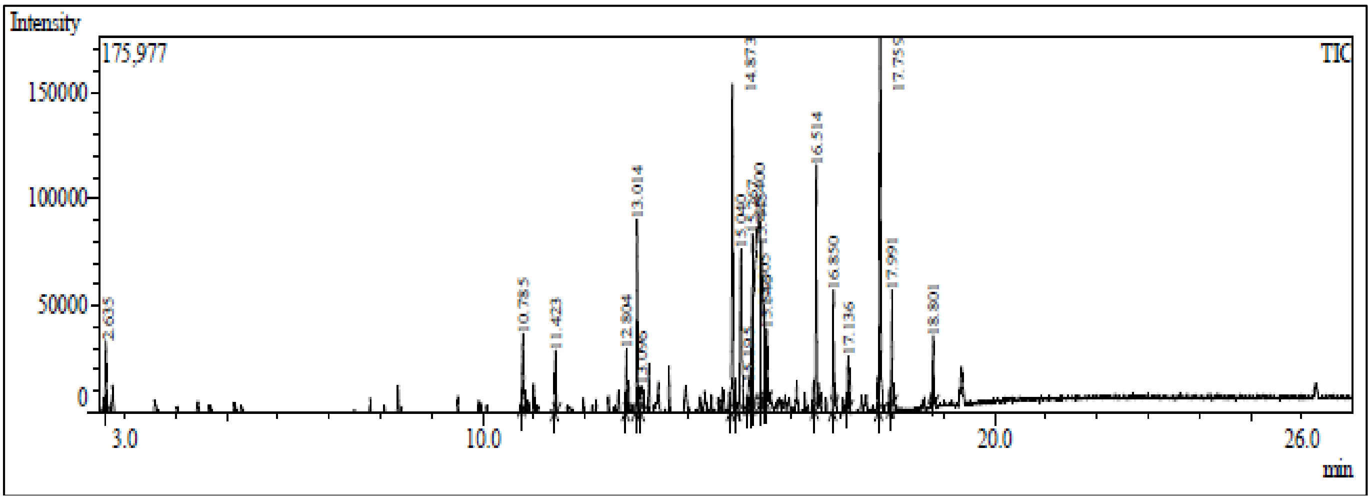

Fig 1: Gas chromatograph of ethanolic leaf extract

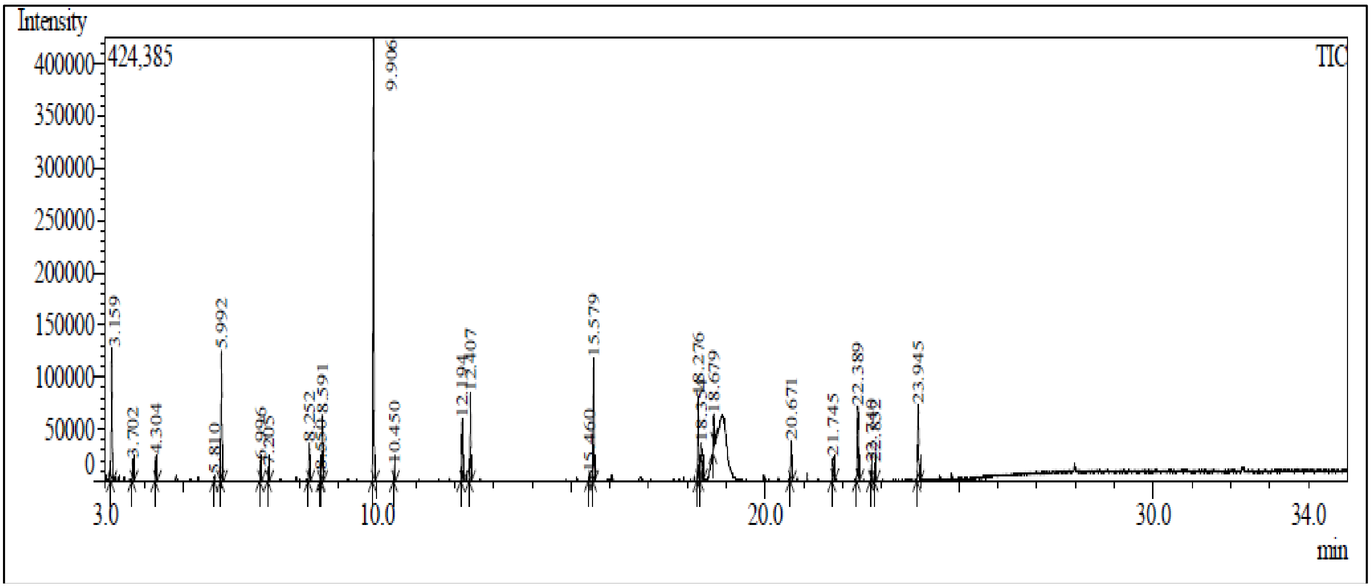

Fig 2: Gas chromatograph of methanolic leaf extract

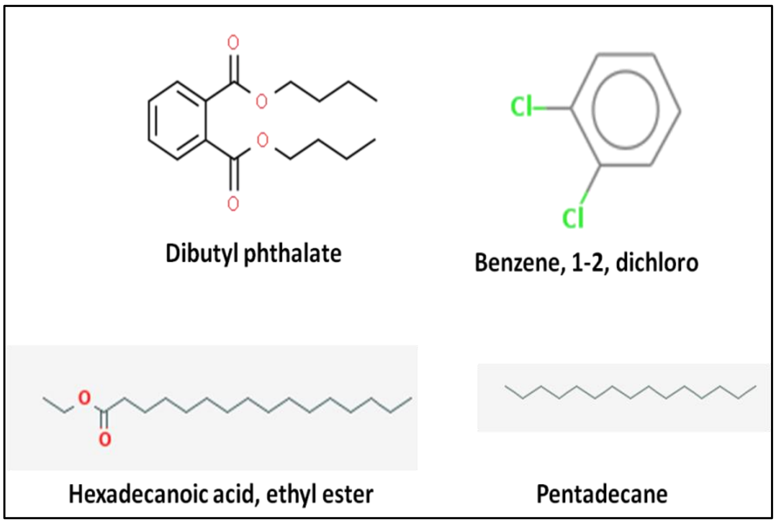

Fig 3: Structure of bioactive compounds with biopesticidal property

Conclusion

GC-MS analysis of solvent extraction of *Strychnos nux-vomica* leaves in ethanol and methanol yielded maximum number of prominent bioactive compounds and hence this method of extraction is found to be promising in many traditional systems of medicine including Unani, Ayurveda, Tibetan, Chinese and Homeopathy. Besides, the seeds, leaves, and barks can be effectively used in many pharmacopeia and developing novel drugs to cure various ailments and plant based botanicals to manage insect pests in eco-friendly manner.

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