The Bioscan AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES 20(3): S.I(3). 1942-1959, 2025 www.thebioscan.com

A dataset on the GC-MS analysis of poisonous plants from fresh, degraded and simulated samples of Strychnos nux-vomica L. and Diospyros montana Roxb.

Poonam Yadav[1*], Swati Dubey Mishra[2], Stalin Nithaniyal[3], M.P. Goutam[4]

[*1]Assistant Professor, Shri Vaishnav Institute of Forensic Science, Shri Vaishanav Vidyapeeth Vishwavidyalaya, Indore, Madhya Pradesh, India.
Orcid id- https://orcid.org/0000-0003-2283-7701
[2]Associate Professor, Shri Vaishnav Institute of Forensic Science, Shri Vaishanav Vidyapeeth Vishwavidyalaya, Indore, Madhya Pradesh, India.
Orcid id- https://orcid.org/0000-0001-7501-7665
[3]Scientific Officer, Botanical Survey of India, Western Regional Centre, Pune, Maharashtra, India. Orcid id- https://orcid.org/0000-0002-9799-1386
[4] Former Professor, Shri Vaishnav Institute of Forensic Science, Shri VaishanavVidyapeeth Vishwavidyalaya, Indore, Madhya Pradesh, India.
Orcid id- https://orcid.org/0000-0002-1094-9506

Corresponding author: Poonam Yadav, E-mail: yadavpoonam077@gmail.com

DOI:10.63001/tbs.2025.v20.i03.S.I(3).pp1942-1959

KEYWORDS

Forensic toxicology, GC–MS, Strychnos nux-vomica, Diospyros montana, toxic compounds, simulated, degraded, boiled plant.

Received on:
06-10-2025

Accepted on:
04-12-2025

Published on:
15-12-2025

ABSTRACT

Toxic plant compound identification in forensic investigations is crucial for deciding the causes of poisoning, specifically in criminal or accidental cases. A comparative Gas Chromatography-Mass Spectrometry (GC–MS) analysis of *Strychnos nux-vomica* L. and *Diospyros montana* Roxb. Collected from different parts of India are presented in this work. This study includes fresh and simulated degraded and boiled samples. The goal of this research was to investigate the chemical profile changes in poisonous phytoconstituents caused by degradation and simulated circumstances, thereby assisting forensic specialists in evaluating biological evidence gathered in deteriorated and boiled phases. The study successfully identified key toxic ingredients such as strychnine and brucine in S. nux-vomica and betulinic acid and phenolic compounds in *D. montana.* These compounds exhibited constituent's pattern in both the degraded and simulated sample types. Our dataset provides a useful reference for forensic toxicology laboratories in cases involving plant-based poisonings, especially when the forensic evidence is retrieved in traces or degraded state.

1. Introduction

In forensic toxicology, the rapid identification and analysis of plant-derived toxins are indispensable. Especially, when the cases involving suspected poisoning through natural toxic compounds. India's rich flora includes several plant species with known toxicological effects, which are often involved in criminal cases, accidental ingestion, or traditional medicine misuse. Phytochemicals that are biologically active and potentially toxic to living things are found in poisonous plants. However, because of their toxicologically significant contents, they are also helpful in treating a variety of disorders. Since these plants may

secretly and peacefully wipe out life, they are regarded as biological weapons. Serious issues or even death may result from their presence (1). *Strychnos nux-vomica* and *Diospyros montana* are plants with known toxic qualities that can be found in both rural and urban settings.

In India *Strychnos nux-vomica* is well known toxic plant belonged to Loganiaceae. Nux vomica is a poisonous plant due to the presence of toxic alkaloids, strychnine and brucine. Both compounds are neurotoxic and competitive antagonists of the glycine receptors on postsynaptic membrane in the spinal cord, brain stem, and higher centers (2). It was found that the lethal doses of strychnine induce convulsions of the central nervous system and death through respiratory or spinal paralysis or cardiac arrest (3). However, the general symptoms of strychnine poisoning with low or moderate doses are including agitation, restlessness, abnormal eye movements, photophobia, stiff joints, myalgia, dark urine and painful muscle spasms (4). Due to the easy accessibility of S. *nux-vomica* seeds and high toxicity, forensic cases are frequently encountered as intentional poisoning and suicide attempts.

The fruit of *Strychnos nux-vomica* L although his rarely used for homicide because to its extremely bitter taste and the quick results that follow injection, it has been documented, Children have been poisoned by ingesting many tonic or purgative tablets containing strychnine. Because dying is so terrible, strychnine suicide is rare. Love philtreuses nux vomica as an aphrodisiac. Because of the overdose, it has resulted in poisoning. There are times when nux-vomica seeds are used to kill Livestock (5). This plant is known to be a highly toxic to human and most domestic animals due to the abundance of highly toxic alkaloids especially strychnine. Since, at small dose, it has diverse clinical applications in traditional Indian (e.g., Ayurveda, Unani and Homeopathy) and Chinese medicines (6).

Plant *Diospyros montana*, although less extensively studied, is a native Indian species that belongs to the family Ebenaceae. Its phytochemical constituents include saponins, tannins, flavonoids, and phenolics, some of which may exhibit toxic effects under certain conditions (7) although not as notorious as *S. nux-vomica*, *D. montana* is of forensic interest due to its traditional usage and potential toxicity, especially when mixed with other substances. The fruit of *D. montana* is poisonous, according to some reports, although it is edible according to others. The water carriers called 'Bhistis' apply the fruits to their hands, which usually develop terrible boils. It is reported that these fruits were used by the Hillman of Travancore to poison fish. Crushed leaves are also used in Chhota Nagpur for this purpose as well (8). Antibacterial activity of leaves and seeds of *Diospyros Montana Roxb.* have been reported by Goutam and Purohit (9) and phytochemical and biological studies on *Diospyros montana Roxb.* have been reviewed by Shubhra Goutam et al (10). Toxicological studies have reported on the toxicity of leaves from this plant, but further research is necessary to determine which phytoconstituents is responsible for toxicity and whether fresh leaves are harmful to fish. Using zebrafish, an initial range finding test was performed to ascertain the LC50 of fresh leaves 1.37 g/0L was found to be the LC50 (11).

In the context of forensic investigations, the analysis of plant toxins is often complicated by the condition of the sample.

Plant material submitted to forensic laboratories is frequently in a degraded state due to environmental exposure, delayed discovery, or deliberate tampering and vomited or excreted samples is extremely difficult because very little amount of fragmented plant. Such plant material will be available and morphological features needed for taxonomic species identification are no longer intact in such samples. The decomposition of plant tissues leads to the breakdown of active phytochemicals, posing a significant challenge to toxicological identification and quantification (12).

Therefore, the creation of comparative datasets involving fresh, simulated degraded samples and cooked samples becomes crucial to validate the stability and detect ability of toxic constituents. Gas Chromatography-Mass Spectrometry (GC-MS) is an advanced and widely accepted analytical method used in forensic science to identify organic compounds in complex its sensitivity and specificity make it ideal for detecting plant-based alkaloids and secondary metabolites, even at trace levels.

This study aims to develop a comprehensive GC–MS-based dataset of fresh, degraded, and boiled leaves and seeds of *S. nux- vomica* and fresh and boiled fruits of *D. montana* collected from various regions of India.

The objective is to establish forensic reference profiles of these plants under different conditions, enabling better identification in real-case scenarios. Such datasets will serve as valuable tools for clinical toxicologists, forensic investigators, and researchers involved in the detection of phytotoxins in medico legal cases.

2. Materials and Methods

2.1 Sampling Site

The plants were collected from different locations including Sagar district of Madhya Pradesh and Sakti districts of Chhattisgarh India. All the plants were authenticated by the Department of Botany, [UGC-DSA/ASIST] Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India and deposited in the herbaria. Plant photographs were documented using Nikon-37. Information on plant habits, habitat, ecology, and other field data were meticulously recorded in the herbarium sheets. Details of plant name, place of collection, and Herbarium accession numbers were provided in the following Table. I

Table- I: Details of plant collection and herbarium

S. No.	Botanical Name	Place of collection	District	State and Country	Herbarium accession number
1.	*Strychnos nux-vomica* L.	Khajurani, Jaijaipur	Sakti	Chhattisgarh, India	BOT/H/04/106/860
2.	*Diospyros montana* Roxb.	Patharia hill, near Dr. Harisingh Gour Central University Campus	Sagar	Madhya Pradesh, India	BOT/H/04/100/04

2.2 Preparation of Samples

Fresh Leaves and Seeds of Plants

Fresh leaves of *S. nux-vomica,* and whole fruits of *D. montana* were selected. The plant parts were washed thoroughly in running tap water and then washed with distilled water to remove impurities. All the samples were air-dried at room temperature and powdered for the analysis.

Fig (A): Fresh leaves of *S. nux-vomica*

Fig (B): Fresh seeds of *S. nux-vomica*

Fig (C): Fresh leaves of *D. montana*

Fig (D): Fresh Fruits with seeds *D. montana*

Simulated Degraded Leaves (*S. nux-vomica and D. montana*)

The simulated degraded leaves of *S. nux-vomica and D. montana,* samples were prepared by keeping them inside the moistened soil for 20 days. Simulated degraded leaves were initially washed with water and then with distilled water. The samples were dried at room temperature and then powered.

Fig (E): Degraded leaves of *S.nux vomica*

Fig (F): Degraded leaves of *D. montana*

Preparation of boiled seed samples (*S. nux-vomica and D. montana)*

Simulated boiled seed samples of plants *S. nux-vomica and Diospyros montana were* prepared by boiling them in water for about 15 minutes. Boiled seeds were cooled and dried at room temperature and then powered

Fig (G): Boiled seeds of *Strychnos nux-vomica*

Fig (H): Boiled fruits with seeds of *D. montana*

Source of all the photo graphs A to H were taken from Ph.D. Thesis submitted by one of the author Poonam Yadav, Shri Vaishnav Institute of Forensic Science, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore, Madhya Pradesh, 2025.

Plants	***Codes used***
Fresh leaves of Strychnous nux vomica	FLSNV
Degraded leaves of Strychnous nux vomica	DLSNV
Fresh seeds of Strychnous nux vomica	FSSNV
Boiled seeds of Strychnous nux vomica	BFSNV
Fresh leaves of Diospyros montana	FLDM
Degraded leaves of Diospyros montana	DLDM
Fresh seeds of Diospyros montana.	FSDM
Boiled seeds of Diospyros montana.	BFDM

Analysis of fresh leaves of *Diospyros montana* have already been reported and published (13)

2.3 Preparation of Methanolic extracts and Hexane extracts

After cleaning the leaves, seeds and fruits samples with tap water, initially these were rinsed with distilled water and allowed to shad dry at room temperature to eliminate any remaining moisture. These dried samples were then ground in to a powder Methanolic extract of Fresh leaves of *Strychnos nux-vomica* (FLSNV), Degraded leaves of *Strychnos nux vomica* (DLSNV), were obtained using methanol (AR Grade) and Fresh leaves of *D. Montana* (FLDM) and degraded leaves of Diospyros montana *(*DLDM) were obtained using methanol (AR Grade Hexane). Hexane extracts of Fresh seeds of *Strychnous nux vomica* (FSSNV), Boiled seeds of *Strychnos nux vomica* (BSSNV) and Fresh fruits with seeds of *D. montana* and *Boiled* fruits including seeds of *D. montana (*BFDM) were subjected to

hexane extraction using AR Grade Hexane to get hexane extract. Powdered samples were placed separately within filter paper to create 20cm by 4.5cm cylindrical thimbles. After being filled with the sample, in each case the thimbles were placed in the cylindrical sample container used in the Soxhlet apparatus and filled with selected solvent as mentioned above. Solvents generated a coloured solution when combined with leaf, seeds and fruit samples the coloured solvent was extracted until, it turned transparent. This extraction took over twenty hours to finish the resulting extracts were collected in 100 ml screw-capped vials separately. The concentrated extracts were kept at 40^0Cfor later use after the solvent was evaporated in a rotary evaporator

2.4 GC-MS experimental Methods

Initially all the extracts were filtered using a Whatman No. 4 and subsequently via a 0.22 membrane filter. The GC-MS analysis to determine the phytochemical components in methanolic extracts of FLSNV, DLSNV, FLDM, DLDM and hexane extracts FSSNV, BSSNV, and FFDM and BFDM were conducted using an Agilent Model 8890 GC System with Single Quadrupole Mass Spectrometer (5977B MSD) analyzer. The instrument's initial temperature for GC-MS detection was set at 75°C, and the hold time was 0.5 minutes. The oven's maximum temperature was 350°C at equilibration time of one minute, temperature programming at a rate of 5°C/min with a value of 180°C, hold three minutes, and hold at a rate of 5°C/min with a value of 300°C, hold time of five minutes. The Agilent 19091S-433Ul (Dimension 30 m x 250 um x 0.25 um) was used with an HP-5ms Ultra Inert, 60°C to 325°C (350°C) medium polar column.

The initial temperature of the HP-5 MS UI was 75 °C. A constant flow rate of 1.2 ml/min of helium gas was employed as the carrier gas. 1µl sample injected (split ratio of 15:1) for every sample separately. Acquisition procedure for the GCMS Agilent 5977 MSD was Quad Temperature 150°C, Ion Source Temperature 230°C, Fixed Electron Energy 70 eV, 3-minute scan interval, and segment from 50 to 600 Da. The solvent delay was 3 minutes, and the overall operating time for the GC-MS was 53.5 minutes. The 50 to 600 (m/z) mass spectral scan range and 1,562 (N=2) scanning speed were chosen. A version of Open Lab CDS 2.5 was utilized to analyze the mass spectra and chromatograms, and the mass detector was a Triple-Axis Detector with a powerful dynode and a long-life electron multiplier.

3. Results and Discussion

GC-MS analysis of Phyto-constituents identified in methanolic extracts of Fresh leaves of *Strychnos nux-vomica* (FLSNV) and *Diospyros montana* Roxb (FLDM)., methanolic extracts of degraded leaves of *Strychnos nux-vomica (*DLSNV) and *Diospyros montana* (DLDM). Hexane extract of fresh seeds of *Strychnos nux vomica* (FSSNV) *and Diospyros montana* (FFDM) and boiled seeds of *Strychnos nux vomica* are (BSSNV*) and* fruits with seeds of *Diospyros montana* (BFDM) were carried out and the results are tabulated in Tables 1to Table8. GC-MS chromatogram is given in Fig.1 to Fig 8.

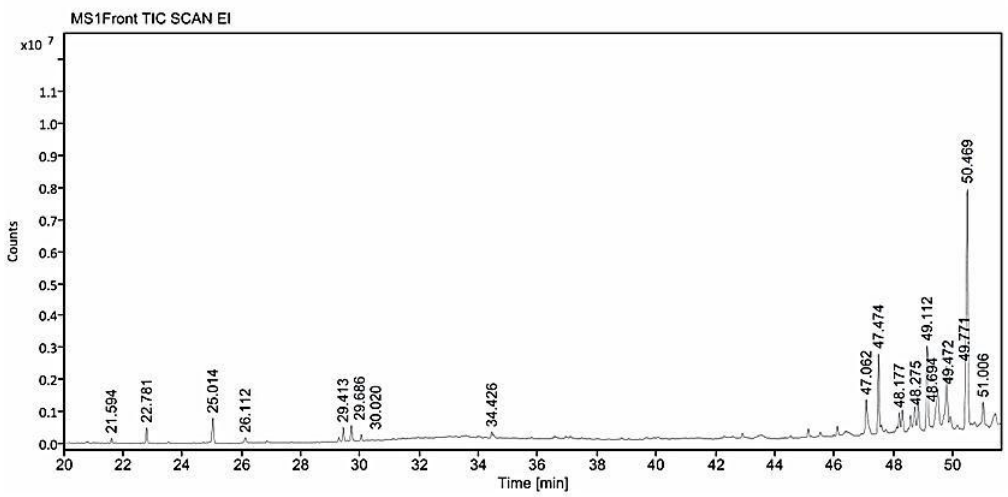

Fig -1: GC–MS chromatogram for methanol extract of the Fresh leaves of *Strychnos nux-vomica* (FLSNV).

Table -1: Phytochemicals identified from the methanol extract of fresh leaves of *Strychnos nux- vomica*.

S. No.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	1-Octadecene	21.594	880	900	3.37	394701.872	0.41	252.486	112-88-9	7715	Hydrocarbon alkene
2	2-Pentadecanone, 6,10,14-trimethyl	22.781	931	951	94.53	1491941.431	1.56	268.477	502-69-2	8676	Alkane
3	Hexadecanoic acid, methyl ester	25.014	922	927	83.02	3048934.334	3.19	256.43	112-39-0	48957	Fatty acid
4	n-Hexadecanoic acid	26.112	837	864	61.43	567429.445	0.59	270.450	57-10-3	9946	Fatty acid
5	10-Octadecenoic acid, methyl ester	29.413	883	887	8.76	1380111.550	1.44	296.5	13481-95-3	20928	
6	Phytol	29.686	872	904	49.32	1683948.780	1.76	296.537	150-86-7	41598	Diterpenoid
7	Heptadecanoic acid, 16-methyl-, methyl ester	30.020	851	871	47.72	573764.841	0.60	298.503	5129-61-3	49021	Ester
8	4,8,12,16-Tetramethylheptadecan4-olide	34.426	769	860	19.38	545993.477	0.57	324.541	96168-15-9	78876	
9	Campesterol	47.062	889	907	62.68	3991800.067	4.17	400.691	474-62-4	7617	Phytosterol
10	Stigmasterol	47.474	909	923	74.94	8338447.361	8.72	412.702	83-48-7	22487	Phytosterol
11	γ-Sitosterol	48.177	847	874	47.94	2142152.791	2.24	414.706	83-47-6	7753	Sterols lipid
12	β-Amyrin	48.275	758	839	20.27	2411647.220	2.52	498.898	559-70-6	214658	Terpenoid
13	4-Campestene-3-one	48.694	747	939	30.21	3272452.024	3.42	398.669	51014-22-3	118904	Steroid
14	α-Amyrin	49.112	787	844	35.58	10896731.441	11.39	426.717	638-95-9	214660	Terpenoid
15	Lanosta-8,24-dien-3-one	49.472	683	830	23.8	7602522.585	7.95	424.7	5539-04-8	39882	Terpenoid
16	Lanosterol	49.771	768	785	23.88	9351512.797	9.78	426.71	79-63-0	262184	Sterol lipid
17	Glutinol	50.469	879	905	87.2	34618393.751	36.19	426.717	545-24-4	234390	Terpenoid
18	Friedelan-3-one	51.006	809	835	47.56	3349793.752	3.50	426.7	559-74-0	38779	Terpenoid

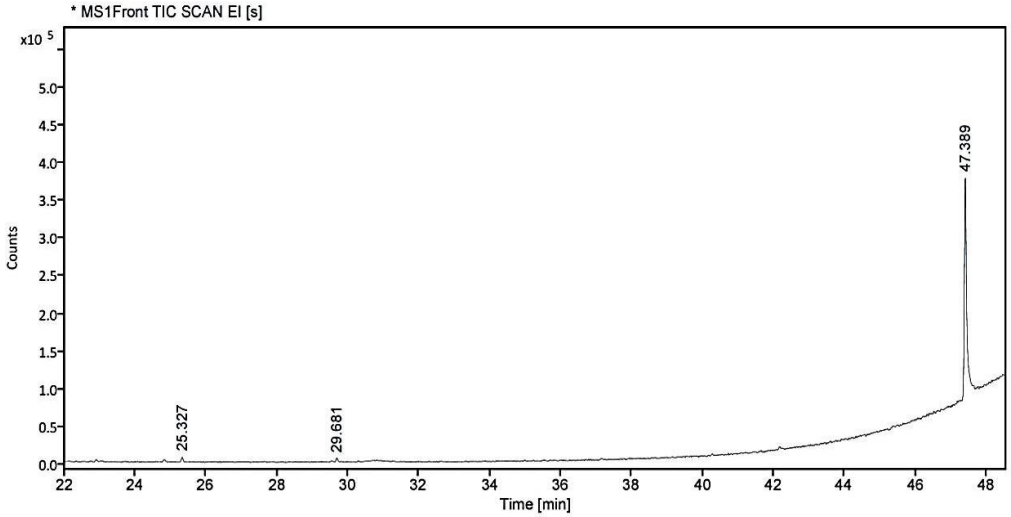

Fig -2: GC–MS chromatogram for methanol extract of the degraded leaves of *Strychnous nux- vomica* (DLSNV).

Table-2: Phytochemicals identified from the methanol extract of Degraded leaves of *Strychnos nux-vomica* (DLSNV)

S. N.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Pentadecanoic acid, 14-methyl-, methyl ester	25.327	615	656	38.54	21348.868	1.52	270.450	5129-60-2	48972	Fatty Acid
2	7-Tetradecenal, (Z)-	29.681	615	676	12.7	14175.453	1.01	210.355	65128-96-3	20786	Fatty Aldehyde
3	Strychnine	47.389	840	862	79.78	1368341.583	97.47	334.419	57-24-9	255205	Monoterpenoid

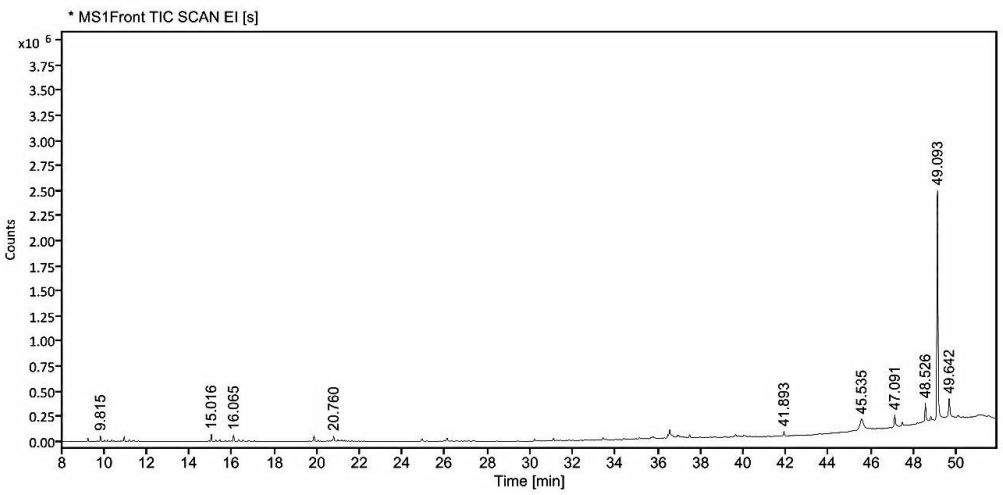

Fig-3: GC–MS chromatogram for hexane extract of the fresh seeds of *Strychnos nux-vomica* (FSSNV).

Table-3: Phytochemicals identified from the hexane extract of fresh seeds of *Strychnos nux-vomica* (FSSNV)

S. N.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Dodecane, 2,6,11-trimethyl	9.815	820	846	7.6	142039.395	1.05	212.42	31295-56-4	27288	Alkanes
2	Heptadecane, 2,6,10,15-tetramethyl	15.016	817	832	6.04	171648.310	1.27	296.6	54833-48-6	27289	Sesquiterpenoid
3	Hexadecane, 2,6,11,15-tetramethyl	16.065	823	833	8.36	189150.263	1.40	282.547	504-44-9	27322	Diterpenoid
4	Eicosane, 2-methyl-	20.760	819	845	7.22	151696.358	1.13	296.574	1560-84-5	26115	Alkanes
5	1-Heptatriacotanol	41.893	667	718	9.55	140139.954	1.04	537	105794-58-9	7819	Alkanes
6	Pagicerine	45.535	659	693	28.97	1260841.625	9.36	380.4	99831-97-7	261105	Alkaloid
7	Strychnine	47.091	733	770	78.31	643216.094	4.78	334.491	57-24-9	255205	Alkaloid
8	β-Amyrin	48.526	744	830	39.7	711189.731	5.28	426.717	559-70-6	214658	Terpenoid
9	α-Amyrin	49.093	916	929	57.64	8604605.552	63.90	426.717	638-95-9	214660	Terpenoid
10	Minocycline	49.642	779	827	74.84	1450270.862	10.77	457.483	10118-90-8	143425	Phenylpropanoid

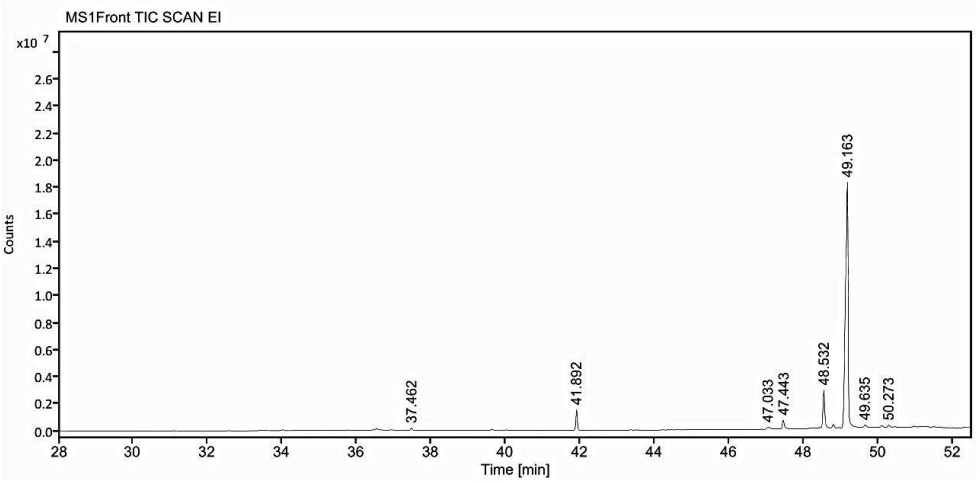

Fig-4: GC–MS chromatogram for hexane extracts of the boiled seeds of *Strychnos nux- vomica* (BSSNV).

Table-4: Phytochemicals identified from the hexane extract of Boiled seeds of *Strychnos nux-vomica* (BSSNV)

S. N.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Erucic acid	37.462	804	837	19.59	582188.921	0.55	338.576	112-86-7	21750	Fatty lipid
2	Squalene	41.892	918	930	57.79	4340446.764	4.13	41.73	111-02-4	38134	Terpenes
3	Campesterol	47.033	776	801	43.52	870149.788	0.83	400.691	474-62-4	7617	Steroid
4	Stigmasterol	47.443	859	891	70.23	2273821.515	2.17	412.702	83-48-7	22487	Steroid
5	β-Amyrin	48.532	935	944	69.69	9725042.279	9.26	426.717	559-70-6	214658	Terpenoid
6	α-Amyrin	49.163	930	931	64.1	85271527.729	81.19	426.717	638-95-9	214660	Terpenoid
7	Minocycline	49.635	753	810	80.13	1264293.794	1.20	457.483	10118-90-8	143425	Steroid
8	Lupeol	50.273	77	734	65.93	698347.729	0.66	426.729	545-47-1	75042	Terpenoid

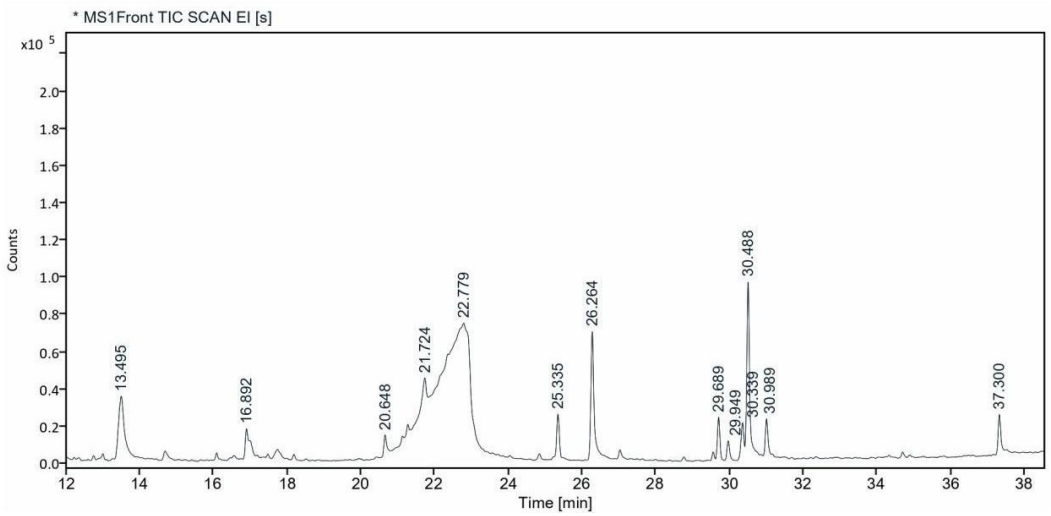

Figure 5: GC-MS Chromatogram of Fresh Leaves of *Diospyros montana* Roxb. (FLDM).

Table -5: Phytochemicals identified from the methanol extract of fresh leaves of *Diospyros montana* Roxb. (FLDM)

S. N.	Molecular Formula	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight	CAS #	Library Id	Phytochemical Class
1	$C_{12}H_{22}O_{11}$	Sucrose	13.495	719	813	56.87	301894.635	12.75	342.30	57-50-1	44727	Carbohydrate
2	$C_{10}H_{12}O_3$	Phenol,4-ethenyl-2,6-dimethoxy-	16.892	753	823	57.88	79601.264	3.36	182.21	28343-22-8	184115	Phenols
3	$C_{10}H_{12}O_3$	(E)-4-(3 Hydroxyprop-1-en-1-yl) -2-methoxyphenol	20.648	703	773	48.31	51839.390	2.19	180.20	32811-40-8	136081	Phenols
4	C6H12O6	L-Glucose	21.724	763	773	8.73	73004.320	3.08	180.16	921-60-8	44368	Carbohydrate
5	C6H14O6	Sorbitol	22.779	775	807	13.66	410763.956	17.35	182.17	50-70-4	44932	Sugar alcohol
6	C17H34O2	Hexadecanoic acid, methyl ester	25.335	820	827	43.84	107205.424	4.53	270.45	112-39-0	48957	Fatty acids
7	C16H32O2	n-Hexadecanoic acid	26.264	872	874	74.98	350762.646	14.81	256.42	57-10-3	9946	Fatty acids
8	C19H32O2	9,12,15Octadecatrienoic acid, methyl ester (Z, Z, Z)-	29.689	771	771	9.31	104181.812	4.40	292.45	301-00-8	53064	Fatty Acyls
9	C20H40O	Phytol	29.949	750	767	34.78	54353.632	2.30	296.53	150-86-7	41598	Diterpenoids
10	C18H32O2	Linoelaidic acid	30.339	821	848	25.82	105693.030	4.46	280.44	506-21-8	35442	Fatty Acyls
11	C19H32O2	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	30.488	881	886	39.39	520594.024	21.99	278.429	463-40-1	53045	Fatty Acyls
12	C18H36O2	Octadecanoic acid	30.989	772	799	65.61	107708.339	4.55	284.48	57-11-4	9948	Fatty acids
13	C19H38O4	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyls)ethylester	37.300	691	761	39.59	100258.420	4.23	330.50	23470-00-0	8214	Glycerolipids

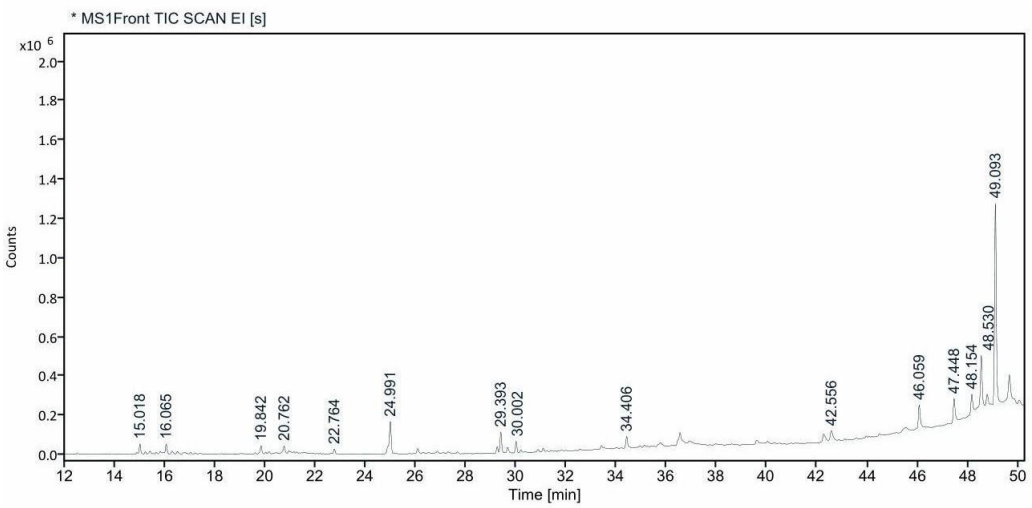

Figure: 6 GC-MS Chromatogram of Degraded leaves of *Diospyros montana* Roxb (DLDM).

Table -6: Phytochemical identified from the methanol extract of degraded leaves of *Diospyros montana* Roxb (DLDM).

S. No.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Heptadecane, 2,6,10,15-tetramethyl-	15.018	840	847	7.62	206267.157	1.84	296.57	54833-48-6	27289	Alkane
2	Heptadecane	16.065	812	827	4.71	629-78-7	1.63	240.475	629-78-7	26125	Alkane
3	Eicosane, 2-methyl-	19.847	807	832	7.97	135261.778	1.21	296.574	1560-84-5	26115	Alkane
4	Hexadecane, 2,6,11,15-tetramethyl-	20.766	803	823	6.12	195571.883	1.75	282.547	504-44-9	27322	Alkane
5	2-Pentadecanone, 6,10,14-trimethyl-	22.760	824	833	76.07	101678.797	0.91	268.477	502-69-2	8676	Alkane
6	Hexadecanoic acid, methyl ester	24.991	894	899	79.45	977419.204	8.74	270.450	112-39-0	48957	Fatty acid
7	9,12,15-Octadecatrienoic acid, methyl ester, (ZZZ)	29.392	832	835	19.35	504976.645	4.52	292.456	301-00-8	53064	Fatty acid
8	Methyl stearate	30.005	861	872	56.22	254309.641	2.27	298.580	112-61-8	48980	Fatty Acid
9	4,8,12,16Tetramethyheptadecan-4-olide	34.412	811	860	50.98	329910.373	2.95	324.541	96168-15-9	78876	Alkane
10	α-Tocospiro A	42.557	748	861	59.96	457640.722	4.09	462.704	601490-40-8	263853	Tocopheroid
11	α-Tocopherolquinone	46.064	629	719	54.86	581015.020	5.20	446.7	-	216289	Phenolic Compound
12	Stigmasterol	47.445	756	825	59.42	646833.602	5.79	412.702	83-48-7	22487	Sterols
13	β-Sitosterol	48.152	736	804	30.84	428721.554	3.83	414.718	83-46-5	7621	Sterol
14	β-Amyrin	48.527	790	859	48.42	1186019.643	10.61	498.898	559-70-6	214658	Triterpene
15	α-Amyrin	49.089	876	907	48.56	4993444.838	44.66	498.898	638-95-9	214660	Triterpene

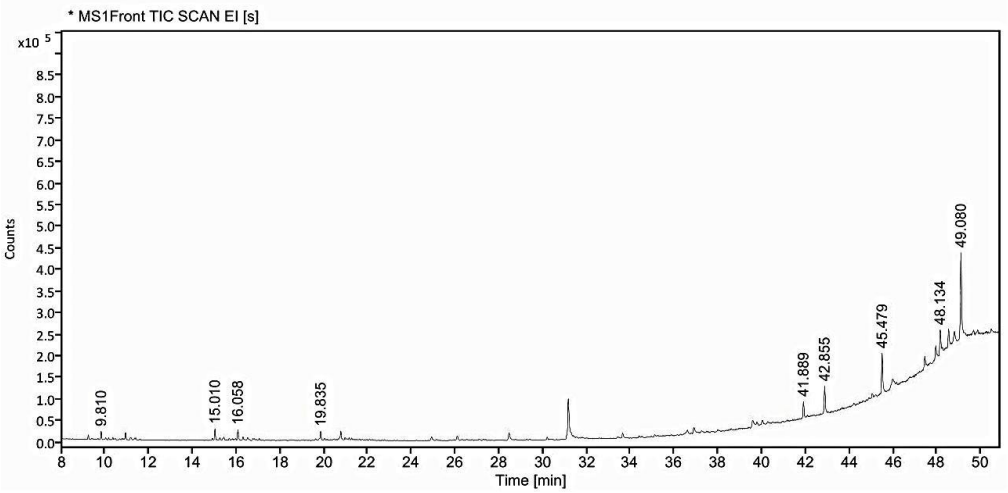

Fig-7: GC–MS chromatogram for hexane extract of the fresh fruit with Seeds of *Diospyros montana* Roxb. (FFDM).

Table-7: Phytochemicals identified from the hexane extract of fresh fruits with seeds of *Diospyros montana* Roxb. (FFDM).

S. No.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Decane, 2,3,5,8-tetramethyl-	9.807	751	810	9.52	49058.364	2.51	198.39	19282 3-15-7	26201	Saturated Hydrocarbon
2	Tetradecane, 2,6,10-trimethyl-	15.008	746	778	6.18	71220.646	3.64	240.5	14905 -56-7	26265	Sesquiterpenoids
3	Hexadecane, 2,6,11,15tetramethyl-	16.058	765	817	6.99	84872.784	4.34	282.547	504-44-9	27322	Diterpenoids
4	Methoxyacetic acid, 4-tetradecyl ester	19.840	716	783	6.23	73379.545	3.75	286.4		18515	Fatty alcohol ester
5	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-y	41.888	634	745	8.97	144618.735	7.40	287.5	66884 -74-0	20766 6	Alkene
6	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	42.857	655	672	8.12	236055.324	12.08	366.707	55282 -12-7	8437	Alkanes
7	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	45.482	641	666	9.9	318427.867	16.29	366.707	55282 -12-7	8437	Alkanes
8	β-Sitosterol	48.133	621	697	14.69	208521.796	10.67	414.718	83-46-5	7621	Phytosterol
9	α-Amyrin	49.077	691	790	53.41	768120.263	39.30	426.717	638-95-9	21466 0	Phytosterol

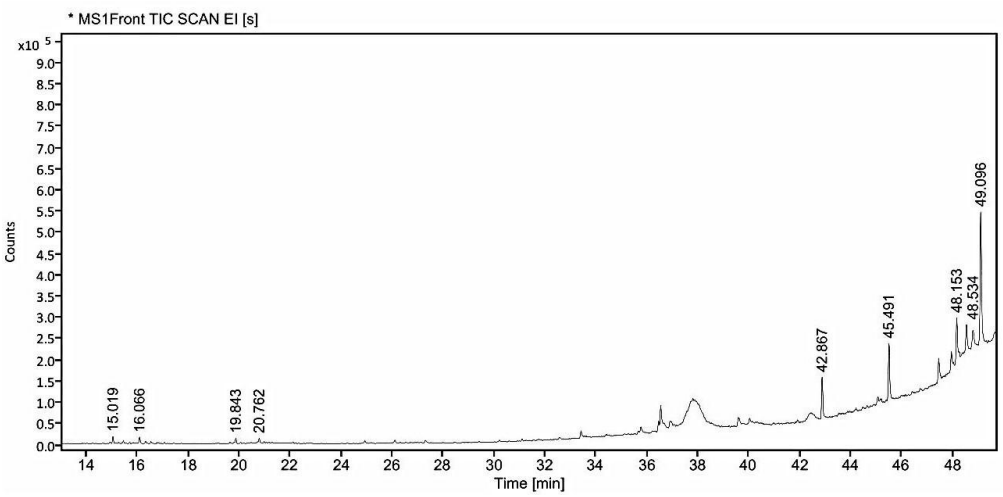

Fig-8: GC–MS chromatogram for hexane extract of Boiled fruit with seeds of *Diospyros montana* Roxb (BFDM).

Table -8: Phytochemical identified from the hexane extract of Boiled fruits with seeds of *Diospyros montana* Roxb (BFDM).

S. No.	Compound Name	RT	Score	Rev. Score	Prob. %	Area	Area %	Molecular weight g/mol	CAS #	Library Id	Phytochemical class
1	Hexadecane, 2,6,11,15-tetramethyl-	15.021	750	770	5.42	43305.415	1.39	282.547	504-44-9	27322	Diterpenoid
2	Pentadecane	16.065	759	792	7.75	43697.599	1.40	212.421	629-62-9	26207	Alkane
3	2-Methyltetracosane	19.846	794	821	18.07	37484.953	1.20	352.68	1560-78-7	26257	Alkane
4	Dodecane, 2,6,11-trimethyl-	20.765	758	803	7.2	44555.256	1.43	212.414	31295-56-4	27288	Alkane
5	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	42.869	710	720	7.51	380973.271	12.21	366.707	55282-12-7	8437	Alkane
7	γ-Sitosterol	48.152	699	727	34.59	402867.584	12.91	432.7	83-47-6	7753	Phytosterol
8	β-Amyrin	48.533	612	713	12.48	297877.790	9.54	426.717	559-70-6	214658	Phytosterol
9	α-Amyrin	49.096	740	819	28.39	1340548.276	42.96	426.717	638-95-9	214660	Phytosterol

This investigation shows that, with the exception of plant *Diospyros montana* damaged leaves, the phyto-constituent composition has changed and decreased when compared to the chemical profile of fresh plant samples of *Strychnos nux-vomica* and *Diospyros montana*. Thirteen phyto-constituents were found in the fresh leaves in this instance, while two more were found following degradation. The amount of elements in fresh fruits containing *Diospyros montana* seeds did not vary. but there was a noticeable shift in the constituents. The fresh samples of S. nux-vomica, however, showed a loss of components. These discrepancies in the outcomes show that phyto-constituents alter as their initial stage varies. These changes could be the result of decomposition breakdown or other causes. However, a number of previous studies have documented a loss of phyto-constituents due to a number of factors, such as thermal degradation (14,15). drying (16,17), boiling (18,19), cooking (20), anaerobic breakdown
(21) and microbial degradation (22).

4. Conclusion

The GC-MS analysis method is clearly a good way to learn about the phyto-constituent profile of plants, as demonstrated by the aforementioned findings. However, GC-MS data of boiled or degraded plants cannot be used to identify the plant because the active constituent that causes toxicity may or may not be present in this altered stage. Therefore, in cases of plant poisoning, appropriate measures must be taken while choosing the antidote during clinical diagnosis. In emergency situations, molecular techniques such as DNA barcoding should be employed if at all possible.

Acknowledgements

The authors are grateful to the head of the advanced Analytical Instrument Facility, Indian Institute of Technology Madras, Chennai, for providing GC-MS facilities for this study.

Funding

None.

Conflict of Interest

The Authors declare that they have no competing interest.

References

1. Gupta VK, Sharma B. Forensic Applications of Indian Traditional Toxic Plants and their Constituents. *Forensic Res CriminolInt J.* 2017;4(1):27-32. DOI: 10.15406/frcij.2017.04.00101.
2. Liu F, Wang X, Han X, Tan X, Kang W. Cytotoxicity and DNA interaction of brucine and strychnine-Two alkaloids of semen strychni. *Int J BiolMacromol.* 2015; 77:92-8. doi: 10.1016/j.ijbiomac.2015.03.017.
3. Philippe G, Angenot L, Tits M. About the toxicity of some Strychnos species and their alkaloids. *Toxicon* 2004; 44:405-16.
4. Maji AK, Banerji P. Strychnos nux-vomica: A Poisonous Plant with Various Aspects of Therapeutic Significance. *J Basic Clin Pharma* 2017;8: S087-S103).
5. Goutam, M.P. and Goutam. S., Analysis of Plant Poisons. Selective and Scientific Books, New Delhi, p284,2006.

6. Daniel M. Medicinal Plants: Chemistry and Properties. Science Publishers, 1st Edition, 2006.
7. Patil, S. M., Jadhav, S. S., & Gaikwad, D. K. (2017). Phytochemical investigation of Diospyros montana for bioactive compounds. *International Journal of Pharmacognosy and Phytochemical Research,* 9(5), 687–692.
8. Chora, R N, Badhwar R.L.and Ghosh S.1965. Poisonous plants of India, Indian Councilof Agricultural Research, New Delhi.
9. Goutam M.P. and Purohit R.M. Antibacterial activity of leaves and seeds of Diospyros montana Roxb. *Indian Journal of Pharmacy,* 1973, Vol. 35, No. 3, 93-94.
10. Goutam, S., Kashyap, M.K. and Goutam, M.P., 2008. Phytochemical and biological studies on Diospoyros montana Roxb., *Journal of Non- Timber Forest Products,* 15(3), pp.151-155. ttps://doi.org/10.54207/bsmps2000-2008-1XDQVT
11. Shridhar NB, Santhosh Kumar CN, Ambika Das, Arun Raj GR,2014. Toxicity study of Diospyros montana in juvenile zebra fish. *International Journal of Pharmacology & Toxicology* 4(2),129-131.
12. Nithaniyal, S., Majumder, S., Umapathy, S., and Parani, M. (2021). Forensic application of DNA barcoding in the identification of commonly occurring poisonous plants. *Journal of forensic and legal medicine,* 78, 102126.
13. Yadav, Poonam & Mishra, Swati, Singh, Kesar and Goutam, M.P. (2024). GC-MS Analysis of Diospyros montana Roxb. Leaves: *A Medicinal and Poisonous Plant. The Bioscan.* 19. 76-82. 10.63001/tbs.2024.v19. i02.S2. pp76-82.
14. Radu, D., Râpeanu, G., Bahrim, G. E., & Stănciuc, N. (2019). Investigations on thermal degradation of phytochemicals from lavender extract. The Annalsof the University Dunarea de Jos of Galati. Fascicle VI- Food Technology, 43(2), 33-47.
15. El Gamal, R., Song, C., Rayan, A. M., Liu, C., Al-Rejaie, S., & El Masry, G. (2023). Thermal degradation of bioactive compounds during drying process ofhorticultural and agronomic products: A comprehensive overview. *Agronomy*, *13*(6), 1580.
16. Yuan, J., Hao, L. J., Wu, G., Wang, S., Duan, J. A., Xie, G. Y., & Qin, M. J. (2015). Effects of drying methods on the phytochemical's contents andantioxidant properties of chrysanthemum flower heads harvested at twodevelopmental stages. *Journal of Functional Foods*, *19*, 786- 795.
17. Al-Qudah, M. A., Onizat, M. A., Alshamari, A. K., Al-Jaber, H. I., Bdair, O.M., Muhaidat, R., ... & Al-Bataineh, N. (2021). Chemical composition andantioxidant activity of Jordanian Artemisia judaica L. as affected by differentdrying methods. *International Journal of Food Properties*, *24*(1), 482-492.
18. Chukwu, O. N., Chisom, I. F., Maureen, C. O., Ekwealor, K. U., &Okeke, C.U. (2015). The effect of boiling on the phytochemical and nutritional content of Rauvol fiavomitoria. *Journal of Global Biosciences,* 4(6), 2561-2568.

19. Ramírez-Moreno, E., Córdoba-Díaz, D., de Cortes Sánchez-Mata, M., Díez-Marqués, C., &Goñi, I. (2013). Effect of boiling on nutritional, antioxidant and physicochemical characteristics in cladodes (Opuntiaficusindica). *LWTFoodScience and Technology*, *51*(1), 296-302.
20. Palermo, M., Pellegrini, N., &Fogliano, V. (2014). The effect of cooking onthe phytochemical content of vegetables. *Journal of the Science of Food and Agriculture*, *94*(6), 1057-1070.
21. Tenney, F. G., & Waksman, S. A. (1930). Composition of natural organicmaterials and decomposition in the soil: V. Decomposition of variouschemical constituents in plant materials, under anaerobic conditions. *SoilScience*, *30*(2), 143.
22. Rogowska-van der Molen, M. A., Berasategui-Lopez, A., Coolen, S., Jansen, R. S., & Welte, C. U. (2023). Microbial degradation of plant toxins. *Environmental microbiology*, *25*(12), 2988-3010.