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Vivo Extraction and Forensic Profiling of Toxic Plant Seeds Constituents via Gas Chromatography Mass Spectrometry

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Abstract

Plants are the outline of human society. Although only some of the chemical components present in the plant are equally crucial for the human body, Sometimes, these compounds accumulate in human body tissues, produce highly toxic elements, and sometimes cause death. Forensic experts need to have a good knowledge of these phytochemicals while performing analysis to interpret toxicology cases. The present study has devised a new extraction protocol for extracting the bioactive components of toxic plant seeds from mice's viscera. The calculated LD50 doses of toxic seed extracts of Jatropha curcus, Datura inoxia, Thevetia neriifolia, Ricinus communis, and Abrus precatorius were given to the mice. A new extraction method was developed for the extracts of the bioactive components after slightly modifying the previously practiced ammonium sulfate method. Gas chromatography-mass spectrometry aids in the conclusive identification of constituents extracted from biological material. Most of the identified compounds were found in the metabolite forms of the phytoconstituents. This new and modified extraction procedure created valid results that helped generate a database of many toxic plant seed constituents and metabolites for its forensic utility.

Keywords: Forensic toxicology, metabolites, bio-active constituents, visceral matrices

1. Introduction

Phytotoxicity is the main concern in phytomedicine and other situations where potentially toxic plants are consumed. Beside many medicinal properties, the plant may also produce many different alkaloids, tannins, glycosides, toxalbumin, etc., that can be too harmful to have toxic effects on humans and livestock [1]. Annual reports of the American Association of Poison Control Centers (AAPCC) suggested around 10–12% of total poisoning cases during 2012–18 were due to toxic plant exposures (https://aapcc.org/annual-reports). Generally, actual figures of fatalities due to plants remain overlooked worldwide, particularly in India. More than hundreds of potentially poisonous plant species belong to over 90 botanical families [2, 3]. Forensic experts may circumstantially face difficulty while examining cases of plant toxicity to screen the specific plant or its derived component responsible for death [4]. Complex chemistry associates a mixture of several hundred different chemical compounds in toxic plants, which makes it easier to understand if good knowledge has been acquired in extracting and identifying metabolites from viscera in forensic cases. In this way, the isolation, detection, and identification of toxic plant constituents could be a tedious job and a subject of reference material when a material is present in trace amounts for analyzing phytochemical compounds from biological samples. The current methods of extracting plant constituents from viscera don’t seem conclusively impressive enough in forensic toxicology. Therefore, the present research report proposes a suitably devised ammonium sulfate extraction method for

alkaloids, tropanes, glycosides, fatty acids, and toxalbumins of five toxic plant seeds of forensic interest from the viscera of mice.

2. Materials and Methods

Collection and sample preparation

Seeds of Jatropha curcus, Datura inoxia, Thevetia neriifolia, Ricinus communis, and Abrus precarious plants were collected from the Rohtak district of Haryana (India). These seeds were washed individually with running water and dried at room temperature for ten days. After powdering the dried seed, about 50 g of powder was extracted with 200 ml of 70% methanol via a continuous hot extraction process for three days. Extracts were evaporated to dryness in the rotary evaporator and stored at 4 °C for further experimentation.

Animal model

Twenty-five Swiss albino female mice (Mus musculus) weighing 25–30 g were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. Mice were kept at the Central Animal House of M.D. University, Rohtak, within standard environmental conditions of a 12-hour dark-light cycle at a temperature of 12 °C and fed a regular diet. The categorization of all mice into five different groups is shown in Table 1. Acute oral toxicity studies were performed per the Organization for Economic Co-operation and Development (OECD) 423 guidelines. The lethal dose (LD50) of test substances was calculated as given in Table 1 [5–9] and dissolved in 1% Tween-80 in saline water [10–11]. The total volume of the test drug administered did not exceed 1 ml per 100 g of animal. Test extracts were administered orally using gastric feeding tubes, and mice were observed continuously for four hours to see if any physical changes were recorded. After mortality, a necropsy was conducted, and visceral samples (stomach contents, liver, kidney, and small intestine) were excised. Viscera were washed with phosphate buffered saline (PBS) and then preserved in a saturated solution of saline water (1 M). A saturated saline solution was given to the control animals, and vital organs were excised after euthanizing them in the CO2 chamber. A new extraction method was developed after modifications to the ammonium sulfate method [12], as shown in Fig. 1.

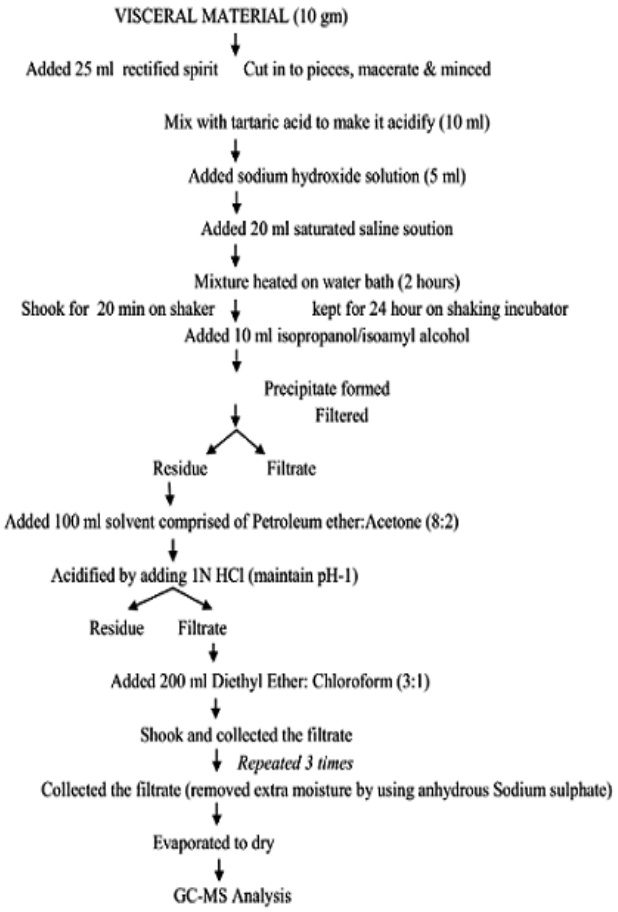


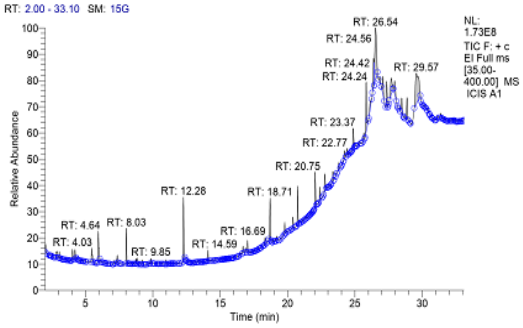
Fig 1-Protocol (modified ammonium sulfate method) for extraction

GC-MS analysis

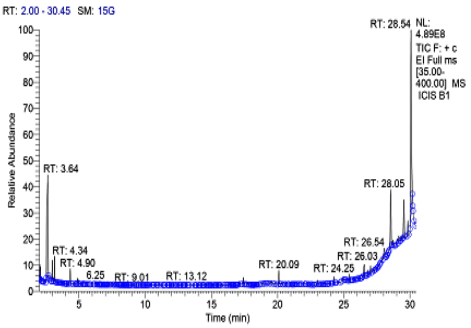
Extracts were dissolved in methanol (GC grade) and filtered through a 0.2-m syringe filter. The TraceTrace GC-MS analyzer (Thermo Scientific Co.) was used for current research at Aryabhata Central Instrument Laboratory, Maharshi Dayanand University, Rohtak, using previously standardized GC-MS conditions [13].

3. Results and Discussion

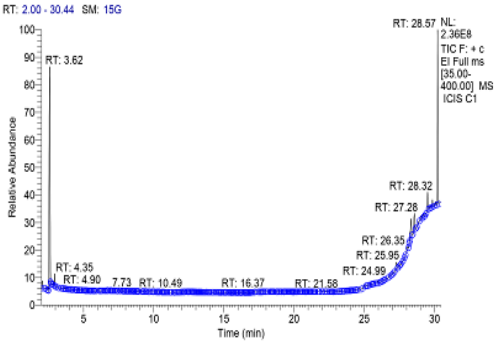
The GC-MS chromatograms and significant compounds detected in extracts from the viscera of mice of test plants treated and control mice are shown in Fig. 2. Identified compounds from the viscera of all five groups of mice and blank-treated control mice are presented in Table 2. The interpretation of the mass spectra of identified compounds was made using the database of the National Institute of Standards and Technology (NIST). The names, retention time (RT), molecular weight (MW), match factors (SI), reverse match factors (RSI), etc., of the components of the test materials were ascertained. The GC separation profiles of extracts from the viscera of different test plant-treated mouse groups had unique characteristics. The presence of metabolites of fatty acids and oil esters was observed in all extracts from the viscera of all five test plant-treated mice. The chromatographic separation profile of Jatropha curcus reported six fatty acids: linoleic, stearic, oleic, palmitic, arachidic, and gadoleic acids [14]. In the current study, we successfully separated different metabolites of these acids, thereby proving the presence of Jatropha curcus in an extract of the viscera of mice (https://pubchem.ncbi.nlm.nih.gov/). Atropine (d,l-hyoscyamine), scopolamine, tropane alkaloid contents derivative of trimethylsilyl, withanolides (lactones), and other tropanes have been reported in chromatographic profiles of extracts of the Datura plant [15, 16]. The GC-MSGC-MSile of Kaner seed oil has registered the presence of oleanitrile, hexadecanenitrile, pentadecane, 8-heptadecene, heptadecane, and octadecanenitrile discussed earlier [17]. The GC-MSGC-MSile of hexane extracts of Ricinus seed oil showed the presence of oleic acid, palmitic acid, stearic acid, methyl ricinoleate, etc. [18, 19]. GC-MS analysis of petroleum ether extract from Abrus precatorius revealed the presence of phytocompounds like n-hexadecanoic acid, [1,1-bicyclopropyl]-2-octanoic acid, 9,12-octadecadienoic acid, ethyl ester, eicosanoic acid, and 2-ethyl-2-methyl-methyl ester [20]. Tetradecanoic acid, palmitic acid, stearic acid, oleic acid, hexadecenoic acid, octadecadienoic acid, pentadecanoic acid, tetradecane-1-ol, tetradecanoic acid, and glycine have been shown in Abrus precatorius seed extracts [21]. The chromatograms of the extracted viscera of mice treated with different toxic plant seeds showed the presence of metabolites of confirmed constituents of selected plants and thereby established the successful extraction of specific plant contents, especially the fatty acids and oil metabolites, from the biological matrices (viscera) of mice. The modified ammonium sulfate extraction method followed by GC-MS analysis proved successful in detecting and identifying potentially toxic plants in mouse viscera. A technique is separately available in the literature [12] for the extraction of alkaloids, glycosides, and toxalbumin, but there needs to be detailed studies to isolate these compounds collectively from visceral matrices. Therefore, the current study developed an extraction method after making some modifications shown in Fig. 1, and extracts were tested for toxic constituents via gas chromatography-mass spectroscopy. The work has successfully identified harmful components from sample matrices, and all chromatogram results were more comparable with their mass spectra, which will be quickly identified from respective plant extracts. One of the drawbacks of this method is the lack of reference standards for toxic groups like alkaloids, cardiac glycosides, and toxalbumin from respective plants.harmful components from sample matrices, and all chromatogram results were more comparable with their mass spectrum will quickly be identified from respective plant extracts. One of the drawbacks of this method is the lack of reference standards for toxic groups like alkaloids, cardiac glycosides, and toxalbumin from respective plants.



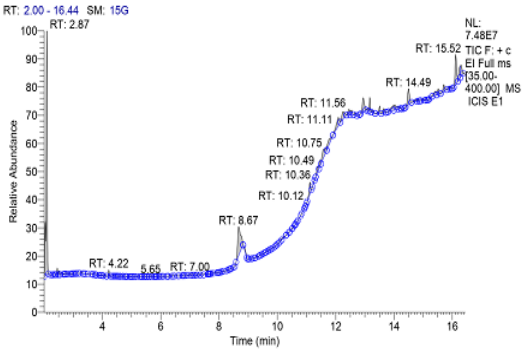
Jatropha curcus



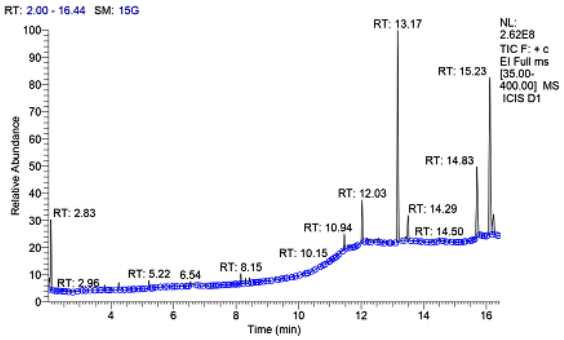
Datura inoxia



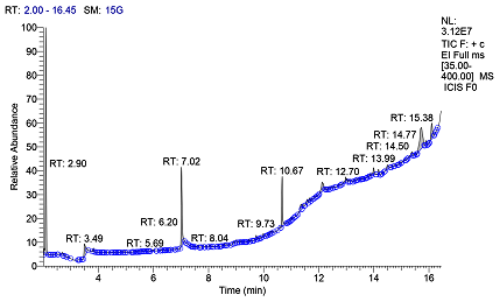
Thevetia neriifolia



Ricinus communis



Abrus precatorius



Blank sample

Fig 2: GC-MS chromatogram of extract of toxic plant and blank control from mice viscera

Table 1- Experiment protocol with dose calculation of selected plant extracts

PE*	VN*	CM*	ATC*	EM*	LD^*_{50}	DC*	References
Jatropha curcus	Physic Nut, Ratanjot	1	Curcin (toxalbumin)	4	2500mg/kg	75mg/30mg	**[5]**
Datura metel	Thorn apple, Jimson weed	1	Hyoscyamine, hyoscine and atropine (Alkaloids)	4	2640mg/kg	79.2mg/30mg	**[6]**
Thevetia neriifolia	Pila/Yellow Kaner	1	Thevetin, thevotoxin, cereberin (glycosides)	4	447 mg/kg	13.41mg/30mg	**[7]**
Ricinus communis	Arand,Castor oil plant	1	Ricin (Toxalbumin)	4	1100 mg/kg	33mg/30mg	**[8]**
Abrus precatorius	Ratti, chirmati	1	Abrin (Toxalbumin)	4	1920mg/kg	57.6mg/30mg	**[9]**

PE* Plant extracts; VN* vernacular name; CM*Control mice; ATC* Active toxic constituents; EM* Experimental mice; LD* Lethal dose; DC* Dose calculated

Table 2- Various compounds separated and subsequently identified from viscera of five toxic plant treated mice and control mice.

RT	Compound identified	CF*	MW*	Nature	Toxicity remark (PubChem)
		Jatropha curcus			
4.03	2-Propanone, 1-hydroxy	$C_3H_6O_2$	74	Aliphatic	Acute toxic
8.03	2,3-Butanediol	$C_4H_{10}O_2$	90	Organic	Toxic
18.71	Di hydroxyacetone	$C_3H_6O_3$	90	Sachhrides	Toxic
20.75	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid	Toxic
23.37	2-Thiazolamine, 4,5-dihydro	$C_3H_6N_2S$	102	Hetrocyclic organic	Toxic
24.24	Benzofuran, 2,3-dihydro	C_8H_8O	120	Coumaric acid	Toxic
24.42	Heptadecanoic acid, 9-methyl-, methyl ester	$C_{19}H_{38}O_2$	298	Methyl ester	No record
24.56	9-Octadecenoic acid,(2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-	$C_{28}H_{44}O_4$	444	Methyl Ester	No record
26.54	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	568	Fatty and aliphatic	Toxic
29.57	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	Omega-7 fatty acid	Toxic
		Datura inoxia			
3.64	3-Heptanone, 5-ethyl-4-methyl	$C_{10}H_{20}O$	156	Ketone	No record
4.34	Methane, oxybis[dichloro	$C_2H_2C_{l4}O$	182	Organic	No record
4.90	Cyclobutene, 2-propenylidene	C_7H_8	92	-	No record
20.09	2,5-Pyrrolidinedione, 1-hydroxy *syn* N-Hydroxysuccinimide	$C_4H_5NO_3$	115	Organic	Toxic
24.25	Dihydro-3-methylene-5-methyl-2-furanone	$C_6H_8O_2$	112	Lipid	Toxic
28.05	Succinic acid, 3-heptyl propyl	$C_{14}H_{26}O_4$	258	Ester of succinate	Irritant

	ester				
28.54	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Ester	Irritant
		Thevetia neriifolia			
3.62	3-Heptanone, 4-methyl	$C_8H_{16}O$	128	Ketone	Toxic
7.73	Ethylbenzene	C_8H_{10}	106	Aromatic alcohol	Toxic
10.49	7-Hexadecene, (Z)-	$C_{16}H_{32}$	224	Fatty acid	No record
21.58	8-Heptadecene	$C_{17}H_{34}$	238	Alkane	No record
28.32	L-Proline, 5-oxo-, methyl ester	$C_6H_9NO_3$	143	Amino ester	No record
28.57	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Ester	No record
		Ricinus communis			
4.22	Dodecane	$C_{12}H_{26}$	170	Alkane hydrocarbon	Toxic
8.67	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	294	Castor ester	Irritant
15.52	5,8,11-Eicosatrienoic acid, methyl ester	$C_{21}H_{36}O_2$	320	Ester	No record
		Abrus precatorius			
5.22	2-Pentanone, 4-methoxy-4-methyl	$C_7H_{14}O_2$	130	Organic	Irritant
6.54	Methyl oxalate	$C_4H_6O_4$	118	Organic	Toxic
13.17	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid	Toxic
14.29	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	Ester	No record
14.83	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	Ester	No record
15.23	Octaethylene glycol monododecyl ether	$C_{28}H_{58}O_9$	538	Non ionic surfactant	Irritant
		Blank			
7.02	Acetic acid	$C_2H_4O_2$	60	Organic	Toxic
10.67	Ethanol, 2,2-diethoxy	$C_6H_{14}O_3$	134	alcohol	No record

CF* Chemical formula; MW* molecular weight

Declarations

Conflicts of interest: None

Author contributions: KK and SB did experiment work and initial draft of the report. RS did have finalized this technical report.

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Ethical Approval: All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of M.D. University, Rohtak, (*Letter No.153-165; dated 17.12.2018).*

Article classification: Technical Report

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