

FORENSIC ANALYSIS AND DETECTION OF POISONOUS PLANTS USING GC-MS, LCMS-MS

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Abstract

Due to the widespread use of poisonous plants as weapons of crime. The accidental ingestion or criminal use of these plant poisons is observed to be increasing day by day; The analysis of poisonous plants has become an important and challenging task. In present, The technique including liquid chromatography coupled with mass spectrometry (LCMS-MS), and Gas Chromatography, Mass Spectrometry (GCMS), were found to be an important technique in the analysis of complex bioactive constituents. The present study was aimed at forensic Detection of poisonous plants from selected Indian plants which are *Calotropis gigantea* (cardenolides like calotropin, calactin, calotoxin, and uscharin), *Nerium oleander* (cardiac glycosides), *Abrus Praetorius* (abrin), *Datura* (alkaloids such as hyoscine (scopolamine), hyoscyamine, and atropine), *Semecarpus anacardium* (bhilawanols , phenolic compounds, bioflavonoids, sterols, and glycosides) by LCMS - Liquid Chromatography Mass Spectrometry and GC-MS (Gas Chromatography Mass Spectrometry) analysis

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Introduction

Chapter I: Introduction

It is only with the earliest man that plant poisons were comprehended, and it was through direct experience that such an ordeal had been an empirical in its form. That then led to the discovery about plants which held toxic chemicals to be used in making hunting and war more effective. The ancient cultures documented the use of poisonous plants, not just for negative purposes such as executions, but also saw their medicinal uses when consumed in moderation. Today, forensic science relies on the identification of these poisons in order to solve cases, witnessing to the ongoing relevance of understanding these complex plant compounds. This history explains the two-sided nature of toxic plants, with a focus on their ability to kill and cure throughout the history of man.

1. History

The history of poisons and poisoning dates back several thousand years. Early poisons were almost exclusively plant and animal toxins, and some minerals. They were used mainly for hunting. Some were used as —ordeal poisons| which means Ingestion of these substances were believed to be lethal to the guilty and harmless to the innocent, for e.g. physostigmine from Physostigmine Veneno sum (Calabar bean), and amygdalin from peach pits. One of the earliest classifications of poisons was done by the Greek physician Dioscorides (AD 40–80) who categorized poisons into 3 groups—Animal, Vegetable, and Mineral An early treatise on plant poisons is De Historia Plantarum, by Theophrastus (370–286 BC). The ancient Indian text Rig Veda (12th century BC) also describes several plant poisons. The Greeks used some plant toxins as poisons of execution. Socrates (470–399 BC) was executed by the administration of hemlock.

In ancient times, the distinction between toxic and non-toxic plant species was learned through trial and error. For malicious purposes, poisonous plants have been used throughout history. A division of poisons into natural and synthetic categories is recognized. The characteristics of an ideal poison, such as its undetectability and painless effect, have been sought. As a method for committing crimes, poisoning has been developed. Even in ancient India, the use of poisoned weapons was known. Plant toxins are still used in crime today. Forensic scientists are aided to crack cases by a complete list of these toxins.

Since there is no good database of these toxins, the following review will attempt to compile. Medicinal plants are traditionally used by ancient cultures because of their medicinal properties. Plant toxins were used by early man to put on arrowheads for hunting. Indian mythological texts contain descriptions of many plants which can be used in their deadly forms. There are many examples in the past where general plant toxins were used against opponents, e.g., Socrates of Rome was condemned to death by poisoning with hemlock (*Conium maculatum*). In 1978, renowned author Georgi Markov is reported to have been killed with ricin, a very potent poison of *Ricinus communis*.⁽⁷⁾ Poisons were familiar to human beings since 4500 BCE and have been employed for different purposes, such as low-dose therapy, antivenoms, and to prepare weapons. Archaeological research has shown that plant poisons (tubocurarine) were used by ancient tribals for weapon preparation more lethal. Toxicology refers to the study of poisons, whereas the root was from the Greek word "taxon," which describes the hunting bow, whereas arrows were initially referred to after "Toxeuma".

1.1 Medicinal plant poison:

It was first found by primitive man, through observation, that certain plants, even though they had the power to do evil, were healing in small quantities. They knew their medicinal worth to some extent in ancient cultures like Egyptians, Greeks, Indians, and Chinese, dosing being very much worked out. A few examples of extremely powerful toxins as medicines are opium poppy for pain, digitalis for cardiac disease, and curare as a muscle relaxant. With the passage of time, such knowledge was maintained and spread by herbalists. As soon as modern science dawned, the active principles of such plants were isolated and identified, and thus the pharmaceuticals came into being. The thin thread between medicine and poison, and dosage making the difference between healing, is mentioned in this history. Dose response in early observations of toxic plants relied on direct observation, whereas toxic species were recognized by early humans based on their adverse physiological effects. Even at the same time, they experienced curing effects in some plants under dosed conditions. These observations were all recorded by ancient civilizations with both poisonous and curative applications. As laboratory technology became better, particularly in the fields of chemistry and botany, scientists began to isolate the individual chemical constituents that were responsible for such activities. The goal of current research is to determine the molecular activity of these compounds with the goal of optimizing their therapeutic potential while reducing their toxic potential.

1.2 Forensic perspective:

Plant toxins are regarded to be evidence of utmost significance in forensic science. Proper identification and detection of toxicants in bio-samples are accorded top priority and achieved

through sophisticated analysis techniques. Cause of death, when poisoning is suspected, depends substantially on agreement between the findings of autopsy and toxicological information. Specialized knowledge is needed in plant poison forensic analysis, the identification of materials recovered from a crime scene, and significant connections are made. Large toxicological profiles of these drugs are deemed critical, and databases are developed for facile recognition. Historical case work, as that involving hemlock, is utilized to give perspective to current case work. The possible employment of plant substances as truth serums will also have to be exhaustively forensically explored. Proper identification of the plant species is deemed to be paramount since each of them harbors different toxins and botanical knowledge is needed. Finally, the forensic approach seeks maximum reliability in identification, toxic effect knowledge, and inter-disciplinary cooperation so that insights from plants can be effectively used in criminal investigations.

1.3 Chemical and Physical properties:

Chemical Properties:

Toxic plants possess an astronomically large number of chemical compounds such as alkaloids, glycosides, proteins, and resins, which are exemplified by advanced analytical techniques. As matrices in plant tissue are compositionally complex, the chemistry of matrices is complex and requires complex extraction and purification protocols for forensic analysis. The chemical composition of alkaloids, which is generally accountable for their acute physiological effect, is generally explained by methods such as GC-MS and HPLC. The chemical structure of glycosides, the sugar moiety and non-sugar moiety, is characterized using separation methods and cyanide tests. The chemical structure of poisonous proteins such as ricin is typically characterized by immunological assays. The breakdown of the chemical structure of plant toxin with time, the need for low detection limits as compared to that chemical structure, and the metabolic breakdown that the substances experience within the body present challenges in forensic analysis. The creation, hence, of extensive libraries of chemical properties has been found to be beneficial. In drug consumption, determination of structure-activity relationships and proper regulation of dosage, in chemical composition, is highlighted for reducing toxicity, thus pointing towards the dual nature of such chemicals in plants.

Physical properties:

Physical traits of poisonous plants are most crucial for pharmaceutical and forensic applications. Macroscopic physical traits, including size, shape, and color, are utilized for plant

species identification of evidence plants at crime scenes and to be used in herbal medicine. Microscopic physical traits, including cell types and vascular bundles, make it possible to identify plant fragments and powdered drugs. Density and texture, physical properties, and volatility and odor, also physical properties, give further clues in forensic examination and field identification. Solubility, a physical property, of plant toxins in various solvents is essential to extract and analyze them, and state, a physical property, influences handling and detection. Physical stability, a physical property, becomes important for medical and forensic purposes. Accurate identification of plants based on these physical features is a critical factor in determining plant material source in criminal cases and safe medicinal use. Knowledge of the physical features is also important in establishing efficient extraction techniques to use in forensic analysis and appropriate forms for medicinal use.

1.4 Uses:

Plant poisons, though harmful, are put to therapeutic as well as criminal uses. Therapeutically, chemically controlled quantities of drugs such as digitalis (in heart disease) or atropine (as a muscle relaxant) are administered. These plants are utilized by indigenous medicine too, albeit with controlled dosing. But criminally, the plants are exploited to induce murder, suicide, and robbery. Forensic science intervenes here, determining the toxins, whether intentionally or not, and what plant it is. Determining the characteristics of the plant is important for medicine and criminal investigation too.

1.5 Forensic Significance:

The medicinal plants have been utilized in the health of human beings since centuries; nevertheless, the medicinal plants can be termed as a double-edged sword due to the fact that the toxic nature of some of the plants can lead to death. The poisonous plants are also utilized for other offenses like homicides, suicides, and war strategies either in one dose or over a prolonged duration of time [153,263]. The toxicological aspects of evidence can be traced to criminal intent by forensic examiners. To identify particular (7). Plant poisons play critical clues in forensic investigations. Scientists employ sophisticated methods to locate and identify such poisons in body tissues. They have to determine if death was caused by poison, and whether it was intentional or accidental. The offense is rebuilt based on how the poison was used and which plant was used. Evidence from plants can connect victims with suspects. The history of plant poisons and history of poisoning must be available. Toxicologists, botanists, and pathologists must collaborate to comprehend these sophisticated poisons.

plant poisons are identified as complex problems. The determination of the poisons in the form of complex mixtures is accomplished by using sophisticated analytical methods. Knowledge of the mechanisms and modes through which the toxins are absorbed, distributed, metabolized, and excreted from the body, and how they lead to harm, is felt to be crucial in the interpretation of results from analysis. Postmortem redistribution of such toxins impacting test results is accounted for. Ground samples and water samples are analyzed to detect environmental contamination by plant toxins. Proper plant identification, in a few instances involving DNA analysis, is viewed as fundamental. Published literature and databases are referred to in order to find information on these toxins. Proper quality control measures are used to provide proper results. Testimony in court, in short terms summarizing results, is given. A scientifically and complex process is basically needed for plant toxins to gain validity in criminal investigations.

1.6 Analysis

Table no 1.6: Some Poisonous Plants

Name of plant	Common Name	Scientific Name	Active principle
Digitalis purpurea	Yellow broom fox glove	Terophularaceae	Digitoxin and digoxin
Plumbago rosea	Raktachitraka	Plumbago rosea	Plumbagin, a naphthoquinone
Cerebra thevetia	Yellow oleander and pila kaner	Apocynaceae	Cardiac glycosides
Calotropis procera	Calotropis sps, and madar, akdo	Apocynaceae	Glycosides like calotropin, calotoxin, And uscharin,
Nerium oleander	White oleander and kaner	Apocynaceae	Cardiac glycosides, particularly oleandrin
Semecarpus anacardium	Marking nut and bhilawa	Anacardiaceae	Bhilawanols, phenolic compounds, biflavonoids, sterols, and glycosides

Dieffenbachia sp.	Dieffenbachia, dumbcane	Araceae	Dieffenbachia seguine
Prunus amygdalus	Almond, baadam	Rosaceae	Oleic and linoleic acid
Nicotiana glauca	Tobacco and tambaku	Solanaceae	Nicotine
Atropa belladonna	Deadly night shade	Solanaceae	Alkaloids, including atropine, scopolamine, and hyoscyamine.
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Datura fastuosa	Thorn apple and datura	Solanaceae	Tropane alkaloids
Erythroxylum coca	Coke, snow	Linaceae	Crystalline tropane alkaloid
Papaver somniferum	Opium poppy and seeds	Papaveraceae	(opium poppy), Analgesic alkaloid morphine
Argemone mexicana	Argemone and sial kanta	Papaveraceae	Alkaloids, notably Sanguinarine and Dihydrosanguinarine
Stychnos nuxvomica	Poison nut and kuchila	Loganiaceae	Alkaloids strychnine and brucine.
Gloriosa superba	Superb lily,	Linaceae	Colchicine

	flame lily and kalihari		
Abrus precatorius	Jequirity, Indian liquorice, guchi or rati	Leguminosae	Abrin, a potent toxalbumin.
Croton tiglium	Croton tiglium, Jamal-gota	Euphobiaceae	Croton, a toxalbumin.
Ricinus communis	Castor bean, erandi	Euphorbiaceae	Ricin
Cannabis sativa or Cannabis indica	a Indian hemp, hashish	Cannabinaceae	Tetrahydrocannabinol
Aconitum napellus	Indianaconite , monkshood and mithazahar	Ranunculaceae	Alkaloids (aconitine, mesaconitine, jesaconitine, hypaconitine)
Aesculus hippocastanum	Horse chestnut, conker	Hippocastanaceae	Aesculus hippocastanum
Alocasia macrorrhiza	Giant taro, elephant ear	Araceae	Saponin
Antiaristoxaria	Upas tree, Antiaris	Moraceae	Cardiac glycosides (cardenolides)
Cerbera odollum	Dabur, pilikirbir	Apocynaceae	Cardiac glycosides
Citrullus colocynthis	Indian wild gourd, bitter cucumber	Cucurbitaceae	Cucurbitacin
Cleistanthus collinus	NA	Euphorbiaceae	Cucurbitacin
Conium maculatum	Poison hemlock	Apiaceae	Coniine

Crotolariaspectabilis	Jhunjhunia	Leguminosae	Abrin
Euphorbia helioscopia	Sun spurge	Euphorbiaceae	Diterpenpids, triternoids.
Lantana camara Lantana	bunch berry	Verbenaceae	Flavonoids, vitamine
Myristicafragrans Nutmeg,	mace tree	Myristicaceae	It's essential oil
Pegnumhermala	Wild rue	ygophyllaceae	Harmaline, harmine
Semecarpusanacardiu m	Marking nut and bhilawa	Anacardiaceae	Anacardic acid, bhilawanol, and semicarpel
Partheniumhysteroph orus	Carrot grass	Compositae	Parthenin

1.7 Our study

Our work targets the most frequent problem of forensic experts: toxic seed identification in criminal samples, particularly when there are restricted or no reference materials available. In response, we compared five toxic plant seeds and used liquid chromatography-mass spectrometry (LC-MS/MS) to thoroughly examine their phytochemical profiles. Based on the considerations of cost-effectiveness and ease of accessibility, we aimed to formulate a procedure that is easy and accurate and can be readily applied to daily forensic practice. We aimed to give forensic toxicologists an easy-to-follow TLC procedure for identification and quantitation of plant toxin seeds so that the cases related to toxins derived from plants could be easily investigated.

Chapter II

Review of Literature

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- **SahuP et al. (2017)** Semecarpus anacardium (SA), Ayurvedic toxic, is Shodhana purified. 1 Pre-shodhit (PSM) and shodhit (SM) SA nut extracts had declining R_f values and urushiol to anacardol conversion from toxic to non-toxic (in SM). 2 Shodhana reduced phenolic and flavonoid content but yield was boosted and toxicity reduced by this conversion.
- **Sindha et al. (2023)** Nature provides numerous drug leads, and hence isolation and identification of such compounds are important against diseases. Abrus precatorius, which is coveted owing to its medicinal properties, was investigated to identify its active constituents. HPTLC was used to identify one compound (APSP-3) having good antioxidant activity. It was also investigated using sophisticated methods such as NMR and mass spectrometry to identify its structure and possible
- **Upmanyu Neeraj et al. (2021)** Herbal drugs are receiving standardization as herbal drugs are now used more than ever. Therefore, in this present study, Semecarpus anacardium's bioactive have been isolated by pharmacognostic analysis. These comprised microscopy, physicochemical screening, HPTLC, NMR, and LC-MS. Flavonoids and alkaloids were suggested through preliminary screening. HPTLC revealed different phytochemicals. Semecarpetin, a bioflavonoid, was the major one obtained through further characterization. Thus, the medicinal significance of the plant is well understood.
- **Sanjeev Kanojiya et al. (2012)** Cardiac glycosides in Calotropis procera regulate the heart contractions. Therefore, scientists developed a rapid LC-MS/MS method for their identification. That employed metal salts to exactly quantify their molecular weights. The method was able to identify calotropagenin and ten analogs found in the leaf extract of the plant. Specific fragments observed in the MS/MS spectra confirmed the presence of the calotropagenin core. Therefore, this LC-MS/MS method is effective for rapid identification of these valuable compounds..
- **Masquelier et al. (2020).** Cardiac glycosides are toxic plant metabolites. Thus, a specific LC-MS/MS technique was established to determine them in herbs and urine. The technique involved a number of important CGs. Herbs were extracted and purified, whereas urine was diluted and cleaned. Quantification limits were low. The technique had good recovery, precision, and linearity. Uncertainty was estimated. Using the technique for Belgian herb

samples showed that they contained no targeted cardiac glycosides. Hence, the technique is appropriate for monitoring the toxins.

- **Zaitsu, et al. (2021).** A Selective LC/MS/MS Method for the Determination of 12 Plant Toxins in Human Serum was developed. The method employed selective column for separation and monolithic SPE column for rapid extraction with analysis time of 30 minutes. The method demonstrated better linearity, accuracy, and precision and extremely low detection and quantitation limits in validation. To demonstrate its utility, the technique was found to detect α -chaconine in readily available serum and protoveratrine A in the poison sample used for the experiment. The technique is the first to detect all these 12 toxins in a single session and allow for earlier diagnosis and treatment of plant poisoning..
- **Suzuki, et al. (2019).** For intentional contamination of drinks, an LC-MS/MS method was developed for 18 plant toxins. Beer, spirits, tea, coffee, and milk beverages were analyzed. The QuEChERS process, modified, was used for extraction and cleanup. The method was accurate, sensitive, and good recovery. Linear calibration graphs (5-200 ng/mL) were suitable for trace level quantitation. This sensitive method can readily identify intentional plant toxin contamination of all beverages
- **Talluri et al, (2021)** Phytochemical screening of medicinal plants is the crux to pharmaceuticals and biotechnology. HR-LCMS is a quick and potent tool for the analysis of complex bioactive molecules. The current research identified the selected Indian medicinal plants *Pongamia pinnata*, *Dodonea viscosa*, *Gardenia resinifera*, and *Gymnospora emarginata*. HR-LCMS ensured the presence of therapeutically active metabolites such as alkaloids (Hydroxycotinine), flavonoids (Apiin, Kaempferol), coumarin (Lomatin), carboxylic acid (Quinic acid), and phenolic compounds (Isoliquiritigenin) in the said plants.
- **Zhao et al. (2014).** The toxic yet medicinally important Aconite plant of Ayurveda and TCM was investigated to chart its root tissue toxic alkaloids. The tissues of three *Aconitum* species were micro-dissected and their compositions elucidated using UHPLC-QTOF MS. The main occurrence of aconitine, mesaconitine, and hypaconitine was in *A. carmichaelii* and *A. kusnezoffii*, restricted to the outer and vascular tissues of big roots. Tissue-specific knowledge can lead to safer use by excluding very toxic areas, ensuring therapeutic effects, and minimizing poisoning

- **Bowerbank et al. (2022)** A twelve-month experiment in Alnwick's Poison Garden created an LC-MS protocol for quantifying 15 toxins in 25 plants. Demonstrated to be precise and accurate ($R^2 > 0.995$), the protocol examined plant material (leaf, bulb, etc.) that estimates child (1-2 years) ingestion hazard using LD50. The most dangerous were *Atropa belladonna* and *Colchicum autumnale*. Careful handling is essential for all plants with child contact avoided when hand-to-mouth ingestion may occur
- **Gupta et al. (2017)** Poisonous plants contain poisonous phytochemicals, some of which are used in the treatment of disease. The drugs (glycosides, alkaloids, etc.) are clandestine bioweapons employed in criminal activity like rape and murder. Free and freely available, they are heaven for killers, added to food many times or employed by contact in India. Identification of causal agents would require a full database of the plants and poisons.
- **Husen et al. (2023)** Poisonous plants, while being toxic, have medicinal activity and are applied in traditional medicine and pharmacology. The article documents prominent poisonous species, which are emphasized with their medicinal potential (antioxidant, anti-inflammatory, etc.) and toxic metabolites. The article gives strong proof of botany, phytochemistry, and pharmacognostic significance. For the sake of biotechnology and pharmacognosy researchers and academics, among others, it illustrates the duality of such plants.
- **Patil et al. (2023)** plant poisons were used in warfare and hunting, e.g., ricin and hemlock. Forensic investigations require correct identification of poisons, frequently hindered by lacunae in plant knowledge. Plant poisons are classified on the basis of chemical nature and mode of action (cardiac glycosides, neurotoxins). Examples are *Abrus precatorius*, *Nerium oleander*, and *Aconitum napellus*. Forensic investigation involves chromatography (TLC, LC, GC) and advanced techniques (MALDI-TOF, LC-MS, GC-MS) for decomposed samples. Toxin signatures facilitate detection. This chapter discusses the use of plant poisons in crime and modern forensic investigation.
- **Boddupalli et al. (2021)** . Plants produce toxins for defense, some of which are fatal to human beings and animals. They were used in the past as weapons, drugs, or in research. Modern science extends our understanding of these "plant biological weapons." This review comments on 12 toxic angiosperms in eight families, their characteristics, distribution, toxicity, symptoms, and medicinal uses. It brings into focus the dualistic function of these plants, from defense to potential harm.

- **Sinha et al. (2017)** Vegetables have various bioactive compounds, some of which are medicinal (digitoxin) and some are toxic. The review integrates toxic plant and compound information (alkaloids, glycosides), which are of value in forensic analysis. It integrates chemical reactions and toxic substances and integrates information from various sources. It selects the most used toxic plants used in criminal activity, which is useful in plant toxin identification..
- **Dauncey et al. (2018)** well-illustrated book chronicles the natural history of the world's most deadly plants. It traces their evolution, toxin production, and biological activity, from hemlock poison to nightshade peril. Medicinal, ritual, and military uses are revealed in color photographs and drawings. The plants' power to ward off predators and affect human systems is explained, with ethnobotanical data and warning tales for professionals and amateurs.
- **Davis et al. (2023)** Most of the plants are poisonous, and they express their poisonous effects on wildlife, animals, and human beings. The toxins range from carcinogenic chemicals such as aristolochic acid in polluted herbal supplements and crops to acutely toxic compounds, such as those in Japanese yew, which is lethal to cattle after a single bite. Others express mild effects such as milk discoloration. This shows the general toxicity of plants, and therefore precautions must be exercised against possible plant poisoning..
- **Aggarwal et al, (2017)** Forensic analysts are frequently confronted with this challenge of individual plant toxin identification in diverse exhibits, which is crucial in cause of death determination. The deaths involve plant toxins like *Abrus Precatorius*, *Strychnos Nux-Vomica*, and *Plumbago Zeylanica*. In an attempt to overcome this, an effective, inexpensive thin-layer chromatography procedure, with a novel solvent system, was established. The procedure effectively detects these toxins in spiked tissues, a valuable forensic laboratory procedure. It is that the identification of these three toxins with a single solvent system simultaneously is novel.
- **Husaini et al. (2022)** Extensive use of plants as food and drugs, especially in the developing world such as Belize, requires toxicological testing. Belize is surrounded by rainforest and has not been reported to have toxicological testing of its indigenous poisonous flora. Testing of 16 poisonous plants from 7 families revealed 13 toxic, 2 non-toxic, and 1 unstudied. Public safety and recommending use of plants require toxicological assessment of plants in Belize.

- **Norris et al. (2015)** Forensic botany involves using plant science to address legal problems and takes advantage of species-specific anatomy and ecology. It is that taxonomic identification is an important element in the analysis of plant evidence in crime scenes. The sub-discipline, new in North America, involves plant anatomy, taxonomy, and ecology. The book 'Forensic Plant Science' deals with methods, case studies, and pedagogy, and includes a microscopic atlas of food plants, and allows for the identification of plant material in crime scenes.
- **Boddupalli et al. (2021).** Plants produce potent toxins for defense, historically used by humans as weapons or drugs. Early humans learned, through trial and error, to differentiate between edible and harmful species. Though modern food sourcing diminishes this knowledge, scientific understanding of plant toxins has advanced. This article reviews 12 angiosperm species, detailing their characteristics, distribution, toxicity, symptoms, and medicinal uses, highlighting their potential as 'Plant Biological Weapons.
- **Napagoda et al, (2022):** Plants have evolved to create defense strategies against environmental stress, insects, and pathogens dating back to the Devonian era, producing secondary metabolites of varied diversity. Alkaloids and glycosides are some of these compounds, which are synergistic in nature, toxic to organisms. Toxicity is varied depending on plant stage, consumption, and susceptibility of victims. These metabolites act on varied molecular targets, impacting ion channels, respiration, and protein synthesis, a reflection of the intricate chemical defenses of plants
- **Tripathi et al. (2021)** Plants have varied toxic chemicals—proteins, alkaloids, glycosides—some of which are acutely toxic. Though numerous, few plants are acutely toxic. Euphorbiaceae and Solanaceae have potent poisons. Such cheap, readily accessible natural toxins are employed in criminal crimes. This review collates plant chemical constituents, toxic doses, and metabolic effects from textbooks and journals. It is that this work is of value to forensic scientists and claims plant poison's forensic relevance.
- **Khajja et al. (2011)** . Poisons, particularly plant weapons, are a fascinatingly sinister subject, utilized in the commission of crimes such as burglary and murder. These weapons are made from poisonous plants or their components. In India, offenders use these poisons in food or through contact on public transport. Botanical weapons, highly lethal, indicate the seriousness of the threat from poisonous plants. It is that the plants are poisonous, and their utilization in crime is a serious matter.

- **Kumari et al. (2022).** GC-MS was utilized to screen five plant seeds' methanolic extracts for the presence of different bioactive compounds. Alkaloids, glycosides, and steroids were present. Forensic toxicology, in this sense, of plant seeds is difficult for quick analysis. Toxic and therapeutic compounds isolated can be useful to forensic toxicologists. Quick identification should be noted.
- **Gotefode et al. (2022)** India's rich flora, while beneficial, contains poisonous plants. Countrymen, who are dependent on local flora, can get exposed to the same. Specific parts of the plant, i.e., seeds and roots, may be poisonous because of alkaloids, glycosides, and other substances. Lovely flowers can be poisonous, and this is a risk. It should be appreciated that knowledge of these plants is important for security.
- **Kiyat et al. (2023).**Lead leaves of *Datura metel* L. were investigated for cytotoxicity and fragmentation by UPLC-MS in this study. Phytochemicals were applied to screen flavonoids, alkaloids, steroids, and saponins. The extract was highly cytotoxic (LC₅₀ 46.16 µg/ml) as ascertained. Baimantuoluoline D (Withanolide steroid, [M⁺] 504.0591 m/z) was identified by UPLC-MS. Since this withanolide is cytotoxic, anti-inflammatory, and anti-microbial, a drug candidate based on **D. metel** essential oil is promising.
- **Sinha et al. (2017)** Plants possess differential chemicals, some of medicinal values, and others toxic. The subsequent review is exclusively devoted to poisonous plant species of forensic significance. The review differentiates toxic compounds such as alkaloids and glycosides and their chemical tests. The paper integrates fragmental literature in order to assist experts. It should be kept in mind that some plants play dual roles. Commonly encountered poisonous plant species utilised as crime weapons are also addressed under it.
- **Malathi et al. (2019)** Phytochemical and secondary metabolism of the precious Indian medicinal plant *Justicia adhatoda* was conducted. Alkaloids, flavonoids, glycosides, etc., occurred in leaf extracts. Phytol, octadecatrienoic acid, and others were shown by GC-MS. The varied phytochemical content suggests the drug-source potentiality of *J. adhatoda*. It has to be understood that the plant is extremely medicinally useful.
- **Hantoosh et al. (2023)** It is difficult for researchers to detect toxic plants due to the high similarity between toxic and non-toxic plants. Conventional techniques of identifying toxic plant can take a long time, hence, automated toxic plant identification system is required. Cascade forward neural network architecture is proposed in this research work to identify toxic plants from leaves. The proposed system is validated on both (toxic leaves/non-toxic leaves) which are captured using smart phone and internet

- **Fikadu et al. (2018).** Researchers found 37 toxic plants in Western Ethiopia which poison livestock including *Datura stramonium* and *Trifolium hybridum*. Livestock owners primarily reported plant poisoning as a result of agricultural expansion along with overgrazing. The livestock consumed these toxic plants through both direct ingestion and indirect contact. According to the research results livestock suffer severe health risks that require governmental assistance for educational programs and deeper scientific investigation. People need to maintain awareness about their environment.
- **Akbar et al. (2020).** A 200-plant review in the book discusses the global medical uses of plants together with their chemical properties and research information until 2017. The research evaluated traditional practices through scientific evidence which illustrates different active components that produce biological effects. The study includes clinical trial data and participant profiles to show how sociocultural aspects influence health outcomes. This book contains details about the adverse effects of substances and their chemical reactions with other medications. The collection of information helps readers comprehend the essential characteristics of medicinal plants.
- **Smith et al. (2022)** The ideas of disease come from the words "poisonous" and "toxic" but casual runs-in with risky plants are only a part of human-plant interactions. These plants were used by humans throughout history as strategic weapons. The ancient Greeks employed hemlock as a weapon of execution while arrow poisons became critical hunting and war weapons. Plants played two primary roles in the course of history that involved their use in ceremonial rituals of shamanic practices and as natural pesticides. Essential knowledge of plant toxins to survive has transformed into contemporary medical and toxicological studies to explore plant-based compounds for therapeutic use. The ongoing threat of unintentional poisonings proves the complex and enduring relationship between humans and the potent plant species growing in their midst. The old tradition of employing plants for scientific study still holds today.
- **Ahmad et al. (2012).** A study team found 45 toxic plant species out of 30 plant families in Islamabad during their plant inventory. The research established twenty plant species that occur naturally and twenty more deliberately planted and five that fall into both categories. A table gives full information regarding each plant's classification and toxic components and how they affect human health. All these species create minor gastrointestinal and nervous disorders but some extremely poisonous plants result in lethal effects. All those living in the region need to study about these plants for their safety.

- **Kocyigit et al.(2023)** Plants also produce natural metabolites as defense chemicals, and they are comprised of toxic proteins. In this review, plant-derived proteins, for instance, lectins and pore-forming toxins, occurring in various plant parts, are discussed. The proteins are promising in biomedical activities, including cancer therapy, but could also be harmful. Their toxic action and mechanisms are studied through research. And thus the review thus touches on their application and eradication, noting that there is a necessity to strike a balance.
- **Khajja et al. (2011)** Toxins, most of all plant weapons, have presented an eerie beauty for centuries. And they are used in the commission of crime. These plant weapons, as the whole plant or their toxic components, are a matter for concern. The criminals use them either in food or by contact, most of all in public transport, in India. It indicates how most readily available and deadly these plants are, and how they are used to inflict injuries.
- **Tamilselvan et al. (2014).** This paper is responsible for toxic medicinal plants used for different treatments. These plants, which have been around for centuries, cure diseases like diabetes and cancer. They possess numerous phytochemicals that are diuretic, purgative, and anti-allergic in nature. This is crucial information for the formulation of pharmaceutical drugs. But let us not forget that these plants, although medicinal, are toxic in nature. Thus, careful study and regulated use are the key."
- **Dwivedi et al. (2021)** Nature supplies all the requirements, and plants have a very significant role. They yield medicine and food, along with toxic secondary metabolites as protection. These metabolites, which are regulated by abiotic and biotic factors, protect plants from herbivory. But human beings and animals, not being capable of distinguishing them, are harmed by them and become ill or even murdered. Therefore, these plants must be familiar. That ignorance is dangerous. These metabolites are organic compounds, and their manufacture is an extremely complicated process.
- **Kulkarni et al. (2021)** HPTLC is a highly sophisticated planar chromatography which is widely used for fingerprinting of medicinal plants, drug estimation, and detection of adulterants. It is more sensitive and analyses quicker than TLC and needs lesser solvent. This article describes HPTLC instrumentation and advantages. And, it also gives examples of drugs and medicines that have been tested with this method. But, one must remember that HPTLC sophistication requires specialized training. Thus, proper usage ensures accurate results.

- **Shivatare et al. (2013)** . HPTLC has an important role in herbal drug analysis development. It's a high-technology qualitative and quantitative analytical technique. The article discusses HPTLC method development and validation under quality control. It discusses how HPTLC deals with the complexity of herbal drugs. But proper validation is needed to develop such standardized methods. So, it's imperative to be familiar with HPTLC. Those methods are applicable to be utilized in a vast range of other applications. And to those applications, HPTLC is of very great significance.
- **Cooper et al. (2013)** The authors of this work are global specialists and it is an overview of general forensic and analytical toxicology. It addresses pharmacokinetics, drug metabolism, and pharmacology and emphasizes more towards drugs of abuse. It's designed as an informational guidebook for working practitioners. And, it's got procedures and concepts of interest to the study of drug effects. But, it's strictly dealing with drugs of abuse, so it's extremely specific. And, that specificity is the reason it's such a treasure trove of information. Whoever reads it open to learning, can learn a great deal. It's a textbook necessity.
- **Gupta et al. (2015).** Plants are full of bioactive chemicals. And, some of them are pretty potent drugs. However, there are some poisonous ones. So, even if there are thousands of them among us, not many of them cause acute, life-threatening disease. Chemical diversity is mind-boggling. Alkaloids, glycosides, and resins belong to this category. These chemicals, while quite possibly beneficial, are also toxic. That is why plant chemistry must be explored. It's a crucial science. And those chemicals found in the plants are quite diverse.
- **Bhattacharjee et al. (2013).** Toxic plants are health hazards, and therefore awareness is necessary. This study was on Rohtak's toxic plants, an area with limited earlier data. It recognized ten toxic plants, including *Thevetia peruviana* and *Datura fastusa*. And, a new TLC solvent system was described for their separation. These findings are of practical utility in forensic science analysis and in public safety. But, it must be stressed, that further research may be required. Those plants are highly dangerous. That is why this research is critical. And that is why such information is of use.
- **Kumari et al. (2018)** Seeds of toxic plants present a substantial danger to livestock, tending to cause serious damage or kill them. And, in their ignorance, such danger is increased. But, the seeds also have use in criminality. This study identified such seeds in Haryana with a new TLC solvent system of separation. These TLC patterns are informative to forensic analysis of suspected cases of livestock poisoning. Remember that awareness and

identification can save lives. And, that is why this research is so critical. Those seeds are very dangerous.

- **Tamilselvan et al. (2014)** These article discusses poisonous medicinal plants used for treatment of different kinds. And, it discusses their historical and current use to medicine. They treat conditions like diabetes and cancer and reveal numerous beneficial phytochemicals. However, they are poisonous, and thus their utilization should be careful. Hence, their properties should be highly familiar. This helps drug companies design drugs. And, it shows how ancient knowledge can give rise to new medicine. They who research such plants have to balance both pros and cons. It is an issue of multiple dimensions.
- **Mtewa et al. (2020)** Throughout this book, phytotoxins are explored in detail, telling their history, present uses and drug discovery potential. Mechanisms, benefits, and risks are all discussed in detail, as necessary for laboratory use. And, phytotoxin management protocols are included. That is why it is very useful. But it does place a strong emphasis on their application in pharmacology. This makes it a useful tool for industry and research staff. Anyone interested in plant compounds can have access to a wealth of material. It is well-organized so that anyone working in the field can utilize it. And that is why it is so valuable.
- **Anywar et al. (2020).** Venomous plants, long shrouded in mystery, have captivated man. And they've played multifarious roles, ranging from assassination to medicine. But their role doesn't end there, and they've been used in hunting, fishing, and the transmission of religious rituals. This chapter distills their old uses and modern drug lead discovery. It also covers their uses as biopesticides and as food additives. That information, once the province of an elite few, is now researched scientifically. And so, such plants are studied. Therefore, their dual role, both harmful and beneficial, is achieved.
- **Dubey et al. (2018)** Plants also emit toxins in defense, and some of them are toxic to people. These toxins, diverse organic substances, are glycosides and alkaloids. But, it is structured differently based on environment. Therefore, a plant's toxic character can vary based on its origin. That is why knowing the chemistry of plants is important. And thus, they must be studied. Those substances can be extremely risky. This makes the identification of plants important. It is a very important field of Study. And due to this reason, this information proves to be useful.
- **Serrano et al. (2018).** Neurotoxic plants, though dangerous, are medicinally useful in small quantities. They, however, produce many intoxications all over the world. Thus, caution is essential since beauty lies in poison. Such plants are considered in the present review. And,

it determines their double nature: remedy and poison. Such plants have chemicals such as anticholinergics and cardiac glycosides. Thus, use with caution is a must. Those unaware might be injured. It is a critical field of study. And that is the reason why this information is worthwhile.

- **Gupta et al. (2019)** This book is a complete guide to veterinary toxicology for students and professionals. It deals with day-to-day toxins, species difference, and diagnostic toxicology. The book offers solutions to dealing with the issues of new farm chemicals and domestic products. It contains 15 chapters discussing topics such as mechanisms of toxicity, poisonings caused by other organisms, and risks posed by drug residues. Each chapter begins with the highlights and concludes with questions and answers. It is a convenient and simple-to-use textbook for learning veterinary toxicology. And it is an excellent reference book for professionals.
- **Patil et al. (2023).** Traditional medicinal plants are also rich in potent toxins. Early man used them for hunting, and Indian writings have instances of lethal use. But, their use is not restricted to killings, like Socrates' hemlock poisoning. And, in 1978, Georgi Markov was assassinated with ricin. These examples reflect the dual importance of plants. They heal, but also destroy. Hence, a study of their nature is of top priority. This context is important in the sense that plants are not just beneficial but poisonous. That knowledge is crucial to medicine and safety. And those myths are very important.
- **Yadav et al. (2021).** Forensic botany is beneficial for interpretation of crime scene, location and nature of crime correlation. This study has taken into account *Datura stramonium*, Bundelkhand local poison. And, it has analyzed the plant under microscopy, TLC, and GCMS. These techniques proved to be effective for plant identification from plant remains. But, GCMS revealed extremely high concentrations of hyoscyne, a lethal poison. Thus, *Datura* is extremely harmful in cases of poisoning. It's interesting, that correct analysis is vital. And those results are vitally important. This is one of the most important results for forensic tests in the region. That plant is very lethal
- **Nasreen et al. (2019).** A quick UPLC-HR/MS separated 20 compounds of leaf extracts of *Calotropis gigantea* such as Deoxy streptomycin and Madecassic acid in a 30-minute water and acetonitrile gradient. The extract showed antimicrobial activities against *Fusarium oxysporum*, *Colletotrichum capsici*, and *Xanthomonas axonopodis* using food poison and agar disc diffusion methods, which suggests its therapeutic value.

Chapter III

Problem Identification

Chapter III: Problem Identification

3.1 **Aim:** The aimed investigation is to analyze the different poisonous plants.

3.2 Problem Findings:

- Forensic analysis and identification of toxic plants constitute a multidisciplinary scientific issue, especially in the case of rising criminal and accidental poisoning by plant toxins. The most crucial issue is the heterogeneous chemical composition of the plants, possessing heterologous groups of bioactive compounds like alkaloids, glycosides, proteins, and resins needing special extraction and purification procedures for proper forensic identification[1].
- Conventional analysis protocols also fall short of the sensitivity and specificity required in the discrimination of structurally similar toxins, particularly in low levels or with complex biological matrices. Moreover, the extensive metabolically degradation of the drugs in the body is a challenge in detection and also requires sophisticated hyphenated technology such as GC-MS and LC-MS/MS for developing sure-shot results[1].
- Still another significant issue is the lack of full standardized protocols and databases for the examination of plant poisons that would hamper effective and periodic forensic examinations. Such a lack is exacerbated by a lack of multidisciplinary skills, especially in botany, to facilitate proper identification of plant evidence from crime scenes. Together, these problems make it imperative that there are strong analytical pipelines, better reference libraries, and more interdisciplinarity between botanists and forensic scientists to better identify and analyze plant toxins in forensic investigations[1].

Chapter IV

Material & Methods

Chapter IV: Material & Methods

This study, conducted in collaboration with Kalinga University, Kotni, Near Mantralaya, Atal Nagar, Naya Raipur (C.G.) and The State Forensic Science Laboratory (SFSL) in Raipur, Chhattisgarh, focuses on the Forensic characterization of poisonous plants. The State Forensic Science Laboratory (SFSL) is a premier institution dedicated to forensic science research and analysis, providing crucial support to the criminal judicial system by means of its advanced forensic services and highly trained professionals specializing in various forensic disciplines. Specifically, this comparative study conducted at the SFSL aimed to analyze the characteristics and detectability of poisonous plants.

4.1 Materials

4.1.1 Glass ware:

- 250 ML Glass beaker {ASGI}
- Borosilicate glass funnel 100mm,
- Test tube (Fisher brand borosilicate glass)
- Watch glass
- Conical flask
- Measuring cylinder
- Petridish (Fisherbrand borosilicate glass)
- GC Vials (Shimadzu 5ml)
- Disposable syringe (Dispovan)
- Microfilter (Micro separations PTFE syringe filter, pore size- 0.2 μ m, diameter 25mm)
- Filter paper
- Motor pistol

4.1.2 Instruments:

- Ultra sonicator (PCI analytics)
- LCMS/MS Triple quadrupole (Thermo Fisher Scientific)
- GCQQMS Triple quadrupole (Shimadzu Nexus GC

4.1.3 Chemicals:

- HPLC Grade Methanol (Qualigens – Thermofisher scientific)
- HPLC Grade Water
- Ethanol (Thermofisher scientific)

4.1.4 Samples:

- Samples has been collected from The outskirts of Chhattisgarh, Raipur.

Table no 3.1.4: Samples

sample	Active principle	Amount	Collection date
Calotropis Gigantea	Glycosides like calotropin, calotoxin, And uscharin	4 to 5 seeds	4 th April 2025
Nerium oleander	Cardiac glycosides	1 seed	4 th April 2025
Abrus precatorius	Abrin, a potent toxalbumin.	4 to 5 seeds	4 th April 2025
Datura	Tropane alkaloids	4 to 5 seeds	4 th April 2025
Seme carpus anacardium.	Bhilawanols, phenolic compounds, bioflavonoids, sterols, and glycosides	1 seed	15 th April 2025

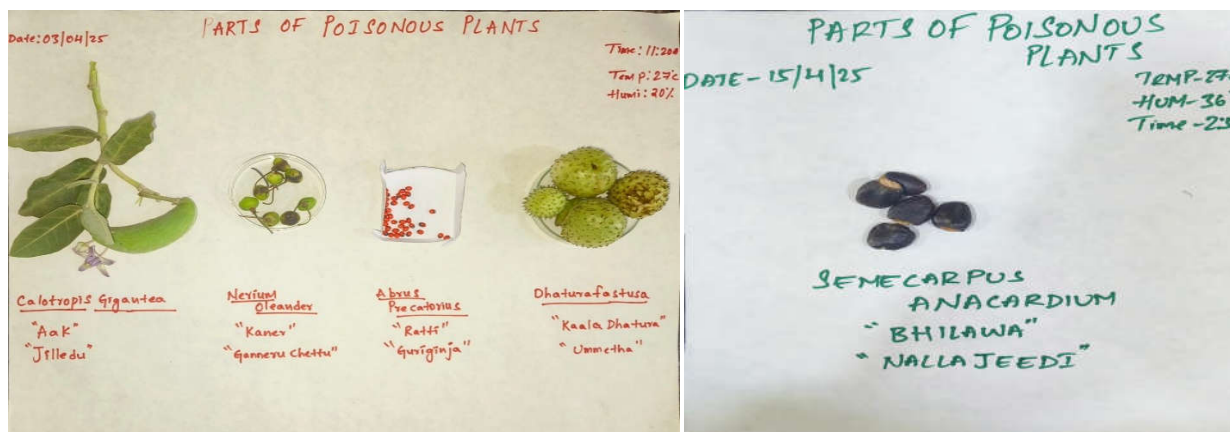


Figure 3.1.4 Poisonous plants samples.

(Source: Self-Analysed)

4.1.5. Extraction

1. We took around 4 to 5 seeds of each sample in motor pistol separately.
2. And crushed the each sample seeds timely diluting it with methanol
3. Later the each sample is transferred to a glass conical flask.
4. In next, each sample conical flask mouth is air tighten with rubber cork
5. Now the samples are left in sonicater for 1 hour.
6. After the sonication the samples are filtered with filter paper
7. Kept the solution still for 2 hours in room temperature
8. The sample containing beaker is totally dried
9. Took a GC vials of 5 ml rinsed with ethanol
10. Same, a disposable syringe of 10 ml also rinsed with ethanol
11. Now the GC vial is filled with 4 ml of ethanol and 1 ml of sample with the help of syringe containing micro filter.
12. Repeated the same process for rest of the sample.
13. The vials containing samples kept in refrigerator for 24 hours .
14. After it samples are ready for the analysis in LCMS/Ms.

4.2 Methodology

4.2.1 Instrument Parameter

1. AUTOSAMPLER PARAMETER

- # of Rinses with Presolvent: 4
- # of Rinses with Solvent(Post): 6
- # of Rinses with Sample: 1
- Plunger Speed(Suction): Middle
- Viscosity Comp Time: 0.2 sec
- Plunger Speed(Injection): High
- Syringe Insertion Speed: High
- Injection Mode: Normal
- Washing Volume: 8 μ L

2. GC PARAMETER

- Column Oven Temp: 80.0 °C
- Injection Temp: 250.00 °C
- Injection Mode: Split
- Flow Control Mode: Column Flow
- Pressure: 65.2 kPa
- Total Flow: 24.0 mL/min
- Column Flow: 1.00 mL/min
- Linear Velocity: 36.8 cm/sec
- Purge Flow: 3.0 mL/min
- Split Ratio: 20.0

Oven Temp. Program

Rate	Temperature °C	Hold Time (min)
-	80.0	3.00
20.00	290.0	7.50

3. MS Parameter

- Ion Source Temp: 250.00 °C
- Interface Temp: 290.00 °C
- Solvent Cut Time: Relative to the Tuning Result
- Detector Gain Mode: 0.96 kV +0.00 kV
- Detector Gain: 0
- Threshold: 0
- --Group 1 – Event 1-
- Start Time: 2.00 min
- End Start: 21.00 min
- Acq. Mode: Q3 Scan
- Event Time: 0.300 Sec
- Scan Speed: 2500
- Start m/z: 32.00
- End m/z: 700.00
- Sample Inlet Unit: GC

Column

Name: SH-Rxi-5Sil MS

Serial #: 1652704

Thickness: 0.25um

Length: 30.0m

Inside Diameter: 0.25mm

1. LC-MS/MS Analysis

LC-MS/MS analysis was carried out using an Ultimate 3000 RSLC system coupled to a TSQ Endura triple quadrupole mass spectrometer. Trace Finder software

(revision 3.2 SP2) was used for instrument control, analysis, data review, and reporting. The LC conditions and gradient are shown in Tables 2 and 3. The LC gradient was optimized to reduce analysis time to 15 minutes, while maintaining good chromatographic separation

LC conditions:

Table : 4.1 LC Conditions

Injection volume	1pl
column' Temperature	250
flow Rate	300 /min
Analytical column	Accucore aQ column, 100 x 2.1 mm, 2.6um μ m
Run time	15 minutes
Tray Temperature	10 °C
Needle cleaning solvent	20% Methanol in water
Sample loop	100 μL
Mobile phases	A : Waler with 5mM ammonium formate and 0.1% formic acid
	B : Methanol with 5 mM ammonium formate and 0.1% formic acid

Table : 4.2 LC Gradient

Time (min)	Flow (mL/min)	A%	B%
0	0.300	100	0
0.5	0.300	100	0
7	0.300	30	70

The TSQ
quadrupole
spectrometer

9	0.300	0	100
12	0.300	0	100
12.5	0.300	100	0
5	0.300	100	0

Endura triple
mass
was operated

in timed-SRM mode. All SRM tracts (parent ion, quantifier ion) were individually tuned for each target analyte by direct infusion of each working standard solution. The mass spectrometer settings are provided in Table:4.3, For convenience and fast method implementation.

MS Settings

Table : 4.3 LC Gradient

Ionization mode	Heated electrospray (HESI)
Scan type	Timed- SRM
Polarity	Positive/Negative switching
Spray voltage for positive mode	3700 v
Spray voltage for Negative mode	2500 v
Sheath gas pressure	30 arbitrary units (Arb)
Aux gas pressure	6 Arb
Sweep gas pressure	1 Arb
Ion transfer tube temperature	325
Vaporizer temperature	350
CID gas pressure	2 m Torr
Cycle time	0.5 s
Q1 resolution (FWHM)	0.7
Q2 resolution (FWHM)	0.7
Chrom filter	3 s

Chapter V

Results & Discussion

Chapter V: Results & Discussion

5.1 RESULTS

A total of 5 samples of poisonous plants were analyzed to detect the active compound of Datura, Seme carpus anacardium, Nerium oleander. Abrus Praetorius, Calotropis. The analysis was performed using Gas Chromatography-Triple Quadrupole Mass Spectrometry (GC-QQQ-MS)

When the samples were examined under GC-QQQ-MS, the GC chromatogram and mass spectra was generated for each sample. They are shown in the figure below

5.1.1 GCMS

5.1.1.1 Datura

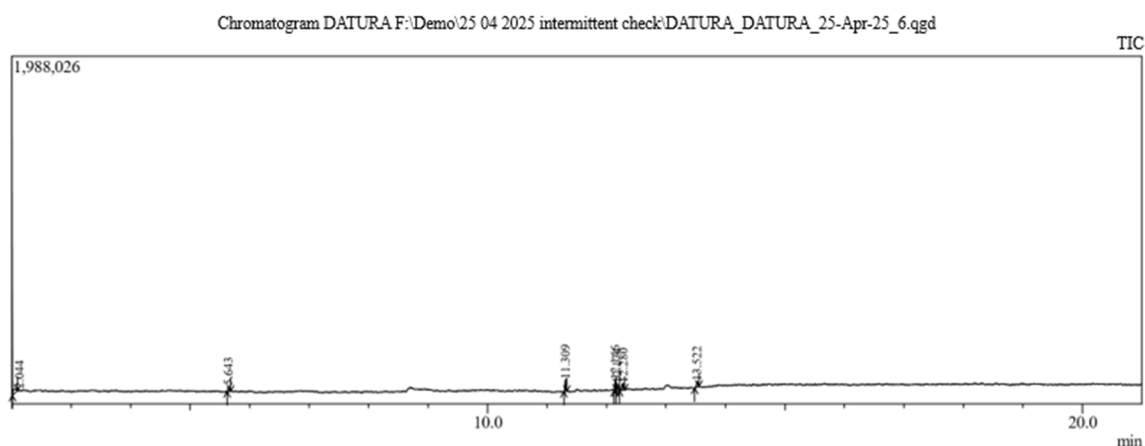


Figure 5.1.1 Chromatogram of Datura
(Source: Self- Analysed)

Interpretation of peak report:

The sample, under the sample ID DATURA, was run on April 25, 2025, with a volume of injection at 1.00 mL being carried out in a high-performance autosampler for repeatability and reproducibility in injections. The peak analysis of some of the major compounds, i.e., Propanoic acid, 3-hydroxy-, hydrazide, (Fig 4.1.2), was at 20.28% of the area, and Tridecane (Fig 4.1.3) was 10.22%. Hexadecanoic acid methyl ester, (Fig 4.1.4) was also found, along with 9,11-Octadecadienoic acid, (Fig 4.1.5) methyl ester (E,E), and 8,11,14-Docosatrienoic acid (Fig 4.1.6) methyl ester with their individual area percentages of 16.30%, 15.34%, and 12.89% respectively. Methyl stearate, (Fig 4.1.7) also came up, adding 11.64% to the overall profile.

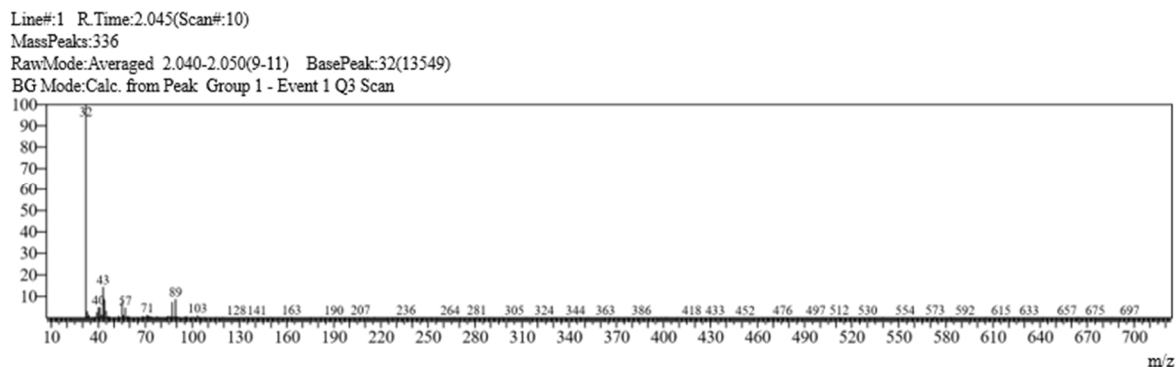


Figure 5.1.1.2 Spectrum base peak of Propanoic acid, 3-hydroxy-, hydra
 (Source: Self- Analysed)

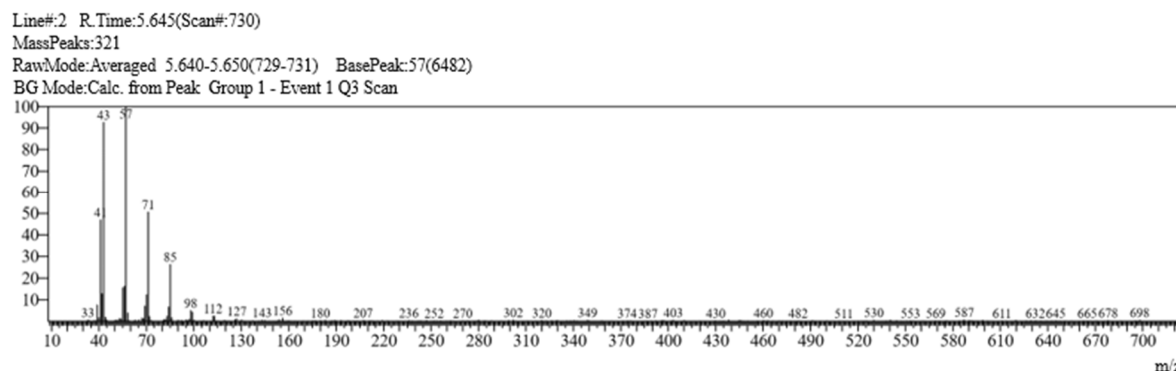


Figure 5.1.1.3 Mass spectrum base peak of Tridecane
 (Source: Self- Analysed)

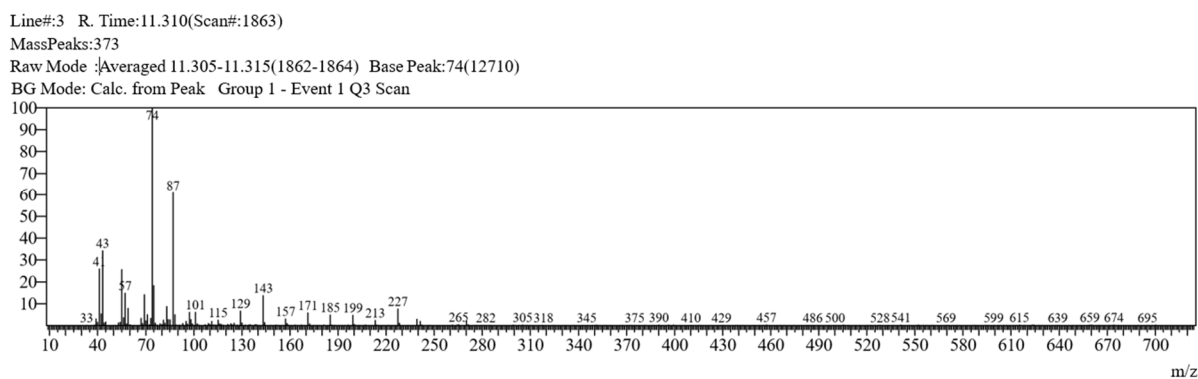


Figure 5.1.1.4 spectrum base peak of Hexadecenoic acid, methyl ester
 (Source: Self- Analysed)

Line#:4 R. Time:12.145(Scan#:2030)

MassPeaks:389

Raw Mode: Averaged 12.140-12.150(2029-2031) BasePeak:67(3641)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

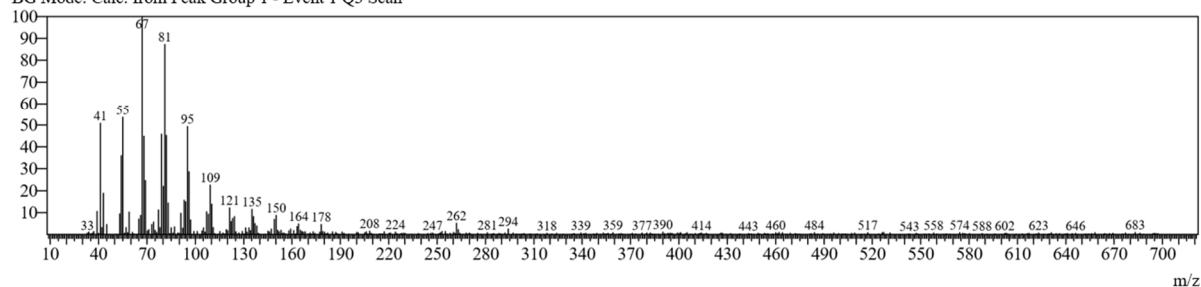


Figure 5.1.1.5 Mass spectrum base peak of 9,11-Octadecadienoic acid, methyl
(Source: Self- Analysed)

Line#:5 R. Time:12.175(Scan#:2036)

MassPeaks:314

Raw Mode: Averaged 12.170-12.180(2035-2037) BasePeak:79(1536)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

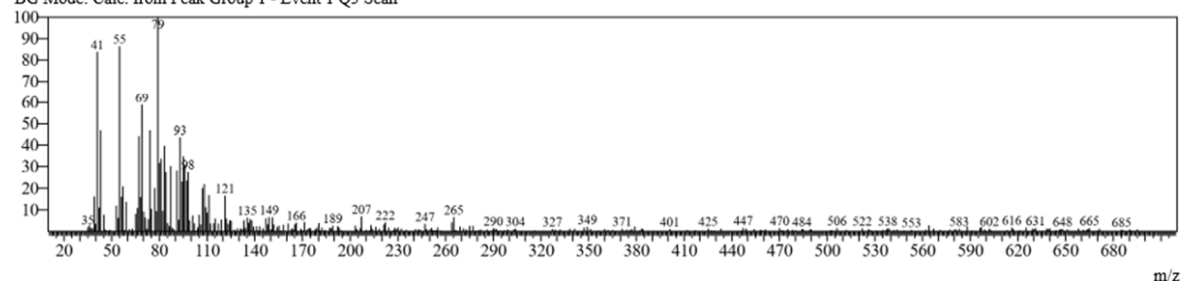


Figure 5.1.1.6 Mass spectrum base peak of 8,11,14-Docosatrienoic acid, met
(Source: Self- Analysed)

Line#:6 R. Time:12.280(Scan#:2057)

MassPeaks:337

Raw Mode: Averaged 12.275-12.285(2056-2058) BasePeak:74(6437)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

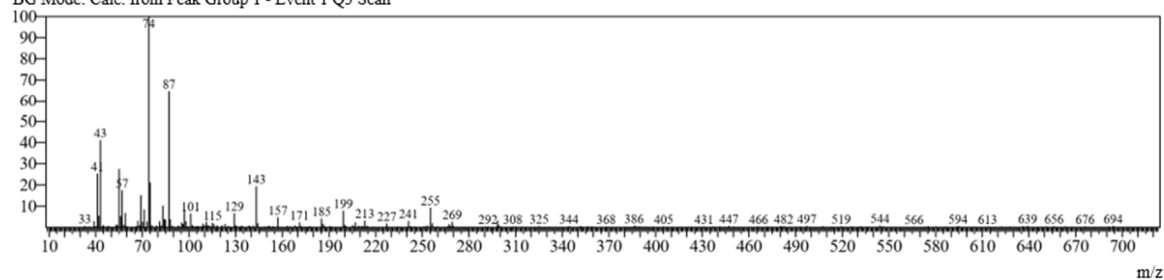


Figure 5.1.1.7 Mass spectrum base peak of Methyl stearate
(Source: Self- Analysed)

peaks were Ginkgol (TMS) (Fig 4.2.5) at 6.02%, 3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl) (Fig 4.2.4) phenol, and (Z)-3-(pentadec-8-en-1-yl) (Fig 4.2.3) phenol, which together also helped give the overall picture of chemicals in the Semicarpus sample.

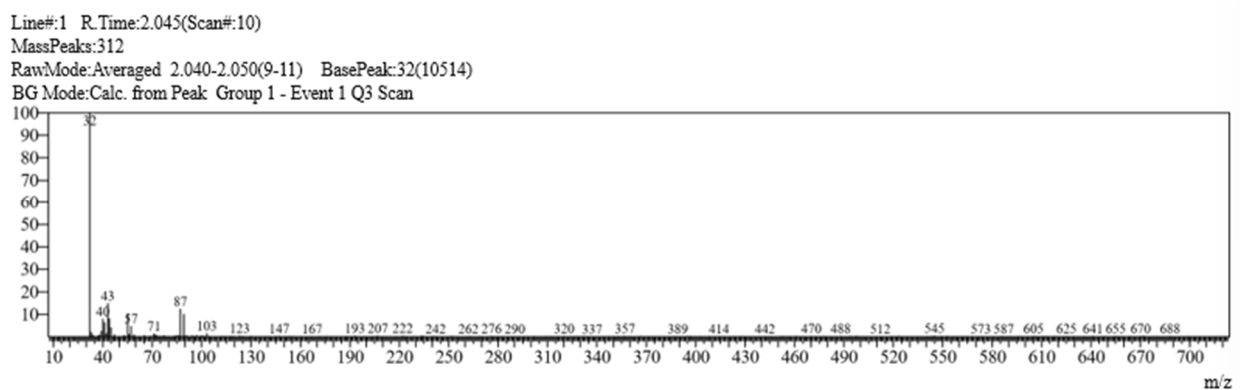


Figure 5.1.2.2 Mass spectrum base peak of Propanoic acid, 3-hydroxy-, hydra
(Source: Self- Analysed)

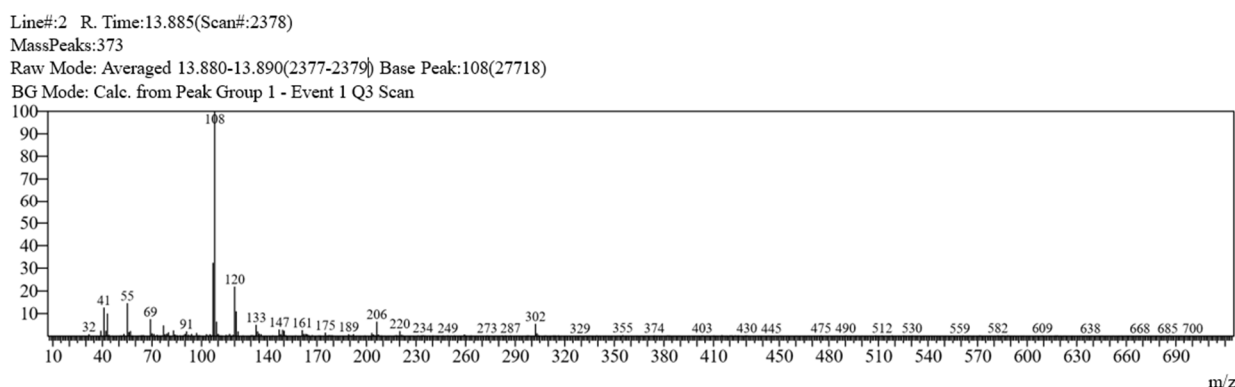


Figure 5.1.2.3 Mass spectrum base peak of 8 (Z)-3-(pentadec-8-en-1-yl) phenol
(Source: Self- Analysed)

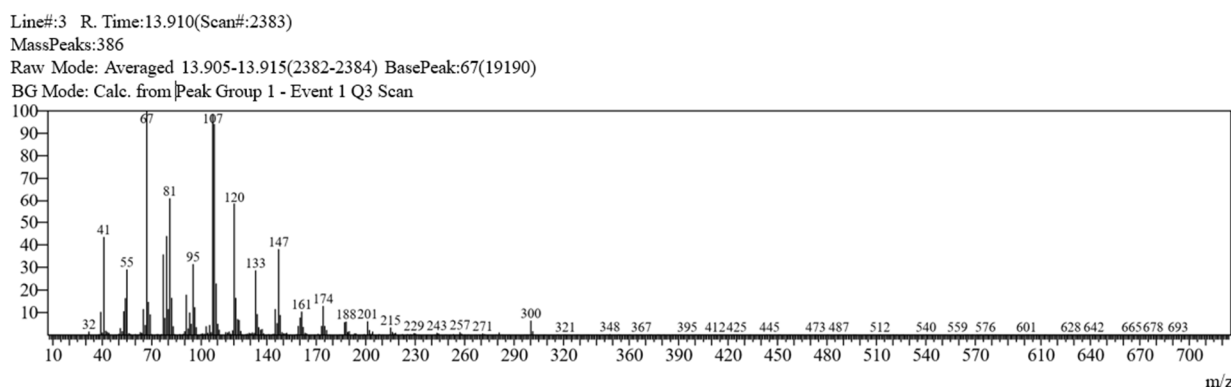


Figure 5.1.2.4 Mass spectrum base peak of Heptadeca-4,7-dien-1
(Source: Self- Analysed)

Line#:4 R. Time:13.985(Scan#:2398)

MassPeaks:521

Raw Mode: Averaged 13.980-13.990(2397-2399) BasePeak:179(149666)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

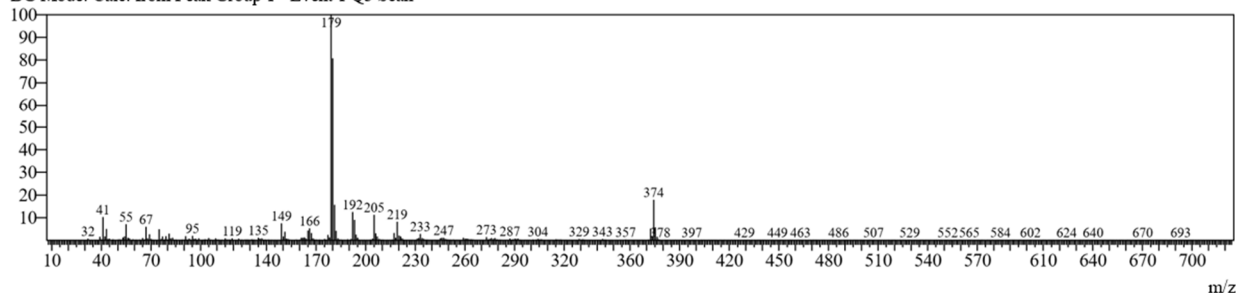


Figure 5.1.2.5 Mass spectrum base peak of Ginkgol (TMS)
(Source: Self- Analysed)

Line#:5 R. Time:14.870(Scan#:2575)

MassPeaks:497

Raw Mode: Averaged 14.865-14.875(2574-2576) BasePeak:123(2861828)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

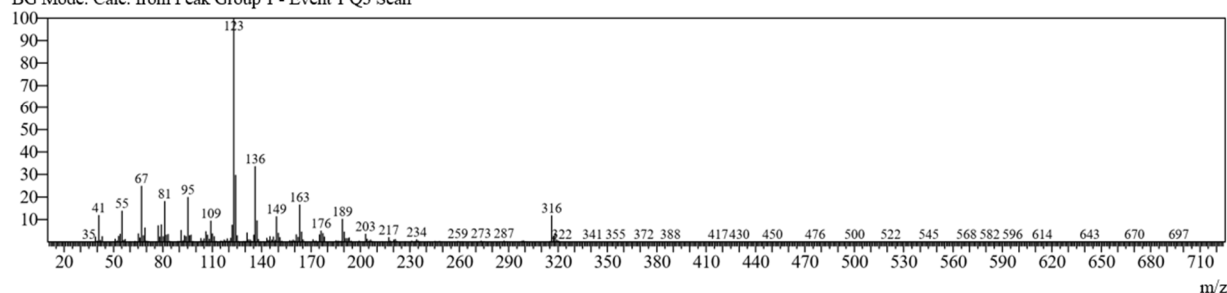


Figure 5.1.2.6 Mass spectrum base peak of Bhilawanol A
(Source: Self- Analysed)

Line#:6 R. Time:15.230(Scan#:2647)

MassPeaks:381

Raw Mode: Averaged 15.225-15.235(2646-2648) BasePeak:123(4784)

BG Mode: Calc. from Peak Group 1 - Event 1 Q3 Scan

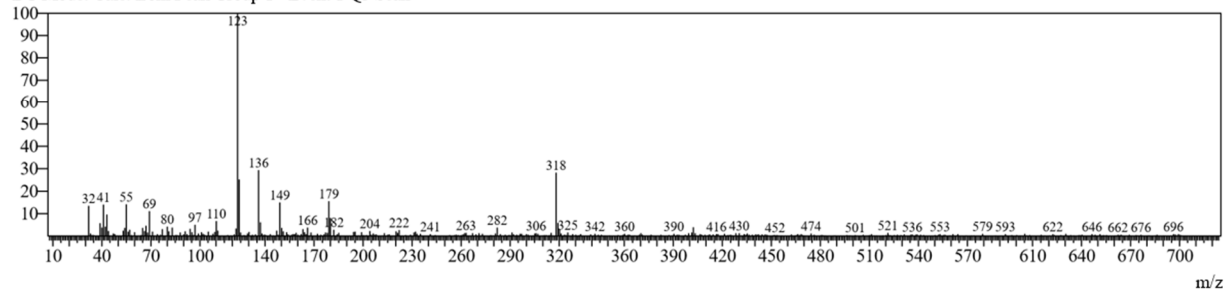


Figure 5.1.2.7 Mass spectrum base peak of Bhilawanol A
(Source: Self- Analysed)

Peak Report TIC										
Peak#	R.Time	Area	Area%	Height	Height%	A/H	Base m/z	Base Int.	CAS#	Name
1	2.043	65556	0.05	21336	0.13	3.07	32.00	10514	24535-11-3	Propanoic acid, 3-hydroxy-, hydr
2	13.885	331833	0.26	254583	1.50	1.30	108.10	27718	501-26-8	(Z)-3-(pentadec-8-en-1-yl)phenol 3
	19190	448252	12-8	3-((4Z,7Z)-Heptadeca-4,7-dien-1						
4	13.986	7676783	6.02	818030	4.83		179.05	149666	0-00-0	Ginkgol (TMS)
5	14.870	118843467	93.22	15443570	91.12	7.60	123.05	2861828	492-91-1	Bhilawanol A
6	15.230	38394	0.03	25292	0.15	1.52	123.05	4784	2764-91-2	Bhilawanol A
		127485594	100.00		16948878	100.00				

Figure 5.1.2.8 peak report

5.2 LCMS - Analysis

A total of 5 samples of poisonous plants were analyzed to detect the active compound of Datura, Seme carpus anacardium, Nerium oleander. Abrus Praetorius, Calotropis. The analysis was performed using Liquid Chromatograph Tandem Mass Spectrometer.

When the samples were examined under LC-QQQ-MS, the LC chromatogram and mass spectra was generated for each sample. They are shown in the figure below

5.2.1. Abrus Precatorius:

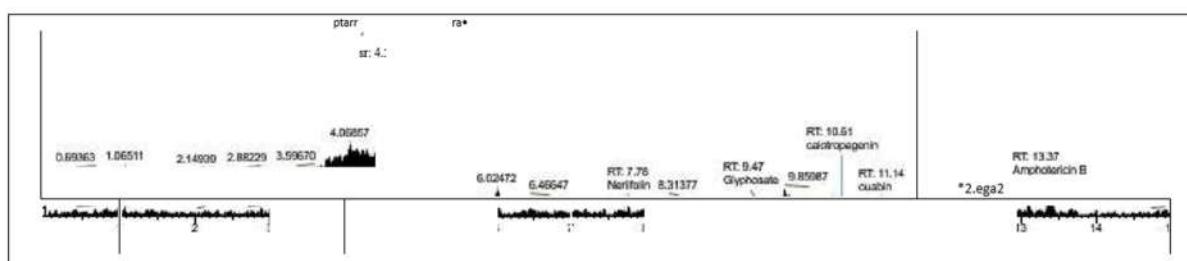
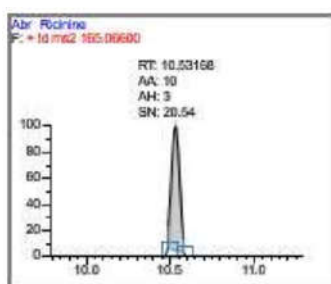


Figure 5.2.1 LC Chromatogram of Abrus precatorius
(Source: Self- Analysed)

Analysis of LC Chromatogram of Abrus Precatorius

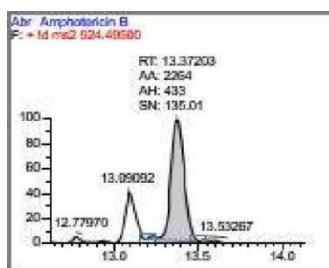
Several peaks at different retention times are seen. Every peak represents a different compound in the sample. To the amazement of the chromatogram, some compounds, i.e., Fig 5.1.9 (Nerifolin RT 7.76 min), Fig 5.1.4 (calotropagenin RT 10.61 min), Fig 5.1.10 Ouabain RT 11.14 min and (Fig 5.1.2) Amphotericin B RT 13.37 min are detected. The presence of the labeled peaks shows that the sample contains a mixture of known chemicals, and identification is made by matching the retention time with standard known compounds. The chromatogram shows the presence of small unlabeled peaks, showing the presence of other, potentially unknown, compounds

5.2.1.1 Ricinin



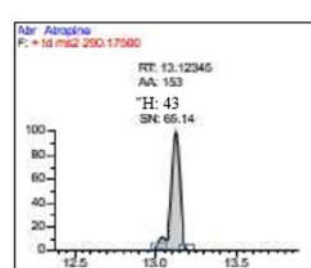
(Source: Self- Analysed)

5.2.1.2 Amphotericin B



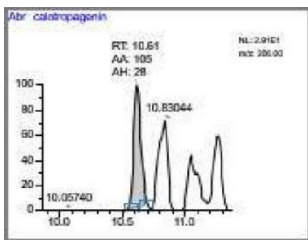
(Source: Self- Analysed)

5.2.1.3 Atropine



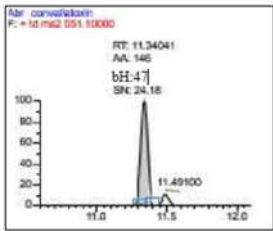
(Source: Self- Analysed)

5.2.1.4 Calotropagenin



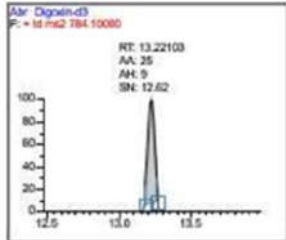
(Source: Self- Analysed)

5.2.1.5 Convallatoxin



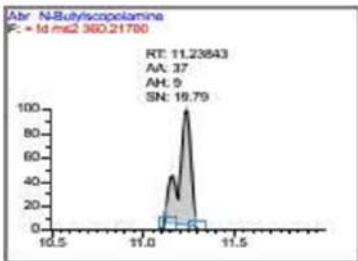
(Source: Self- Analysed)

5.2.1.6 Digoxin-d3



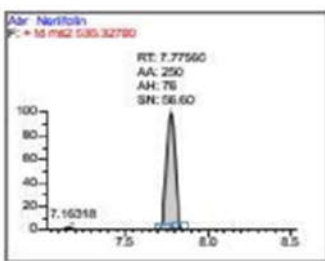
(Source: Self- Analys)

5.2.1.7 N-Butylscopolamine



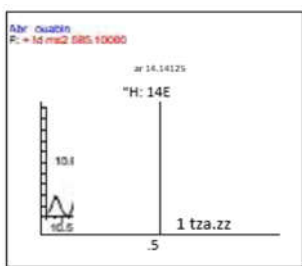
(Source: Self- Analysed)

5.2.1.8 Neriifolin



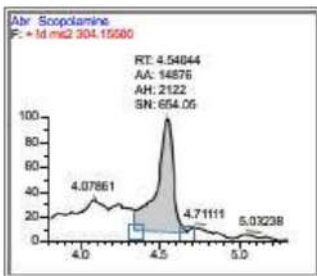
(Source: Self- Analysed)

5.2.1.9 Ouabin



(Source: Self- Analysed)

5.2.1.10 Scopolamine

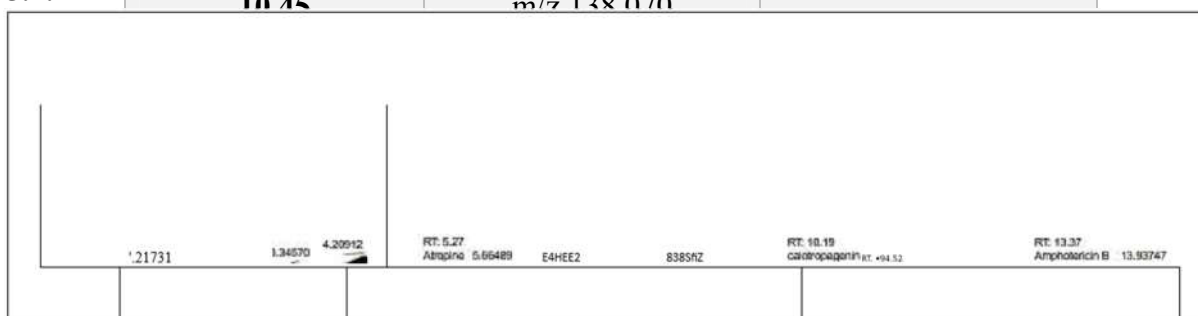


(Source: Self- Analysed)

Table 5.2.1.11 Analytical Results: Compound Identification by RT and m/z

Retention Time (Min)	ION	Detected Compound
13.37 13.37	m/z 355.11. m/z 429.130	Amphotercin B
13.12 13.12	m/z 93.70 m/z 124.130	Atropine
10.61	m/z 206.000	Calotropagenin
11.34	m/z 369.200	Convallatoxin
0.00	m/z 635.100	Digitoxin
0.00	m/z 651.100	Digoxin
13.22	m/z 654.100	Digoxin-d3
11.24 11.24	m/z 103.070 m/z 194.160	N-Butylscopolamine
7.78 7.78	m/z 339.300 m/z 375.380	Nerifolin
11.16 11.16	m/z 94.080 m/z 152.210	N-Methylscopolamine
0.00	m/z 373.290 m/z 433.290	Oleandrin
11.14	m/z 403.100	Ouabin
10.53 10.45	m/z 82.050 m/z 138.070	Ricine

5.2.2



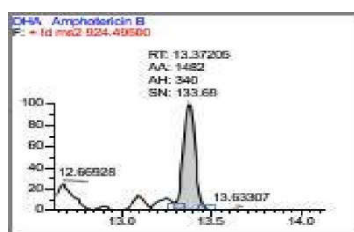
Datura

Fig 5.2.2 Shows LC Chromatogram Datura
(Source: Self- Analysed)

Analysis of LC Chromatogram of Datura

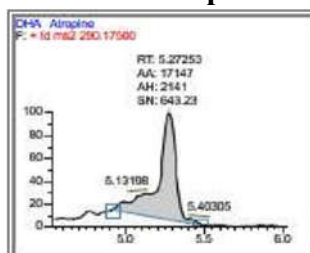
Peaks of interest observed are (Fig 6.1.2) Atropine at 5.27 minutes, (Fig 6.1.3) calotropagenin at 10.19 minutes, and (6.1.1) Amphotericin B at 13.37 minutes, setting the sample to contain these pharmacologically active compounds. Other low-level peaks of smaller retention time at 1.34 and 4.20 minutes could be other minor constituents or impurities. Sharpness of resolution of these peaks indicates the success of the chromatographic technique in separating mixed complex systems.

5.2.2.1 Amphotericin B



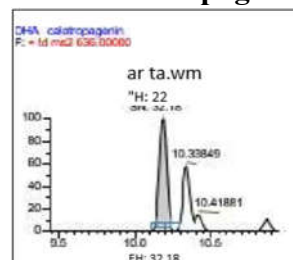
(Source: Self- Analysed)

5.2.2.2 Atropine



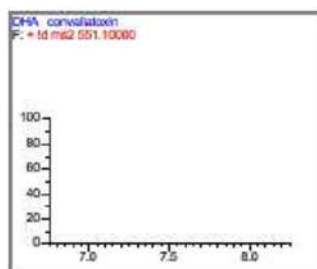
(Source: Analysed)

5.2.2.3 calotropagenin

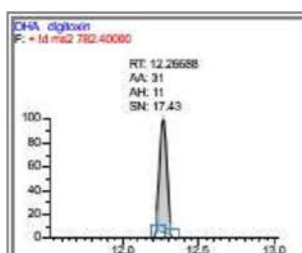


(Source: Self- Analysed)

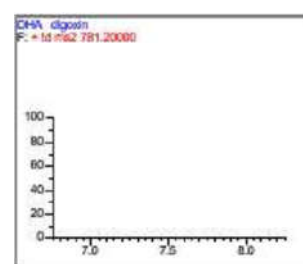
5.2.2.4 convallatoxin



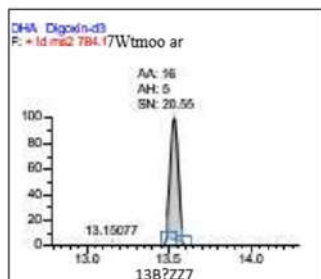
5.2.2.5 digitoxin



5.2.2.6 digoxin

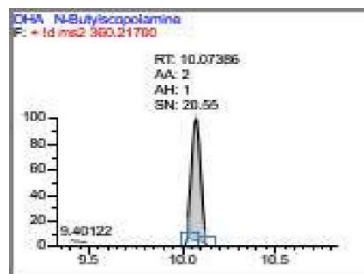


(Source: Self- Analysed)

5.2.2.7 Digoxin-d3

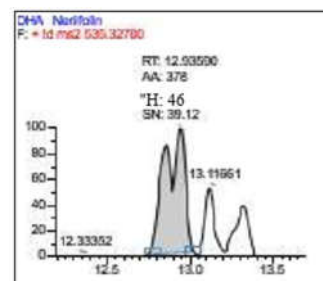
(Source: Self- Analysed)

(Source: Self- Analysed)

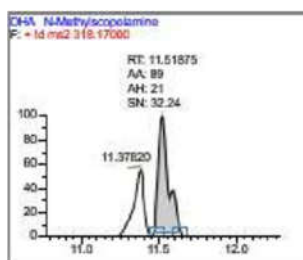
5.2.2.8 N-Butylscopolamine

(Source: Self- Analysed)

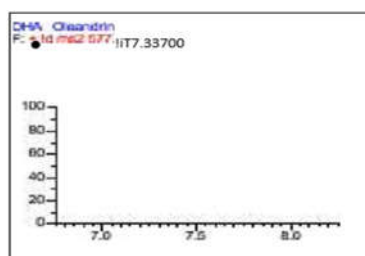
(Source: Self- Analysed)

5.2.2.9 Neriifolin

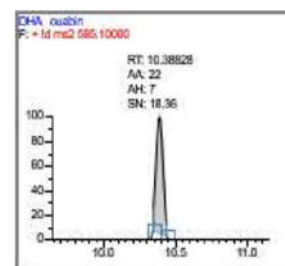
(Source: Self- Analysed)

5.2.2.10 N-Methylscopolamine

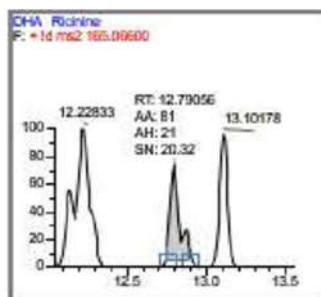
(Source: Self- Analysed)

5.2.2.11 Oleandrin

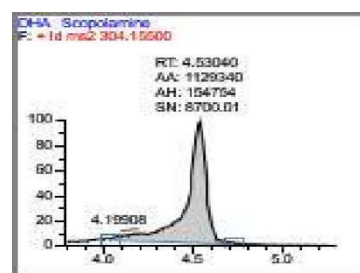
(Source: Self- Analysed)

5.2.2.12 Ouabin

(Source: Self- Analysed)

5.2.2.13 Ricinine

(Source: Self- Analysed)

5.2.2.14 Scopolamine

(Source: Self- Analysed)

Table 5.2.2.15 Analytical Results: Compound Identification by RT and m/z

Retention Time (Min)	ION	Detected Compound
13.37 13.37	m/z 355.110 m/z 429.130	Amphatercin B
5.27 5.27	m/z 93.070 m/z 124.130	Atropine
10.19	m/z 206.000	Calotropagenin
0.00	m/z 369.200	Convallatoxin
12.27	m/z 635.100	Digitoxin
0.00	m/z 651.100	Digoxin
13.53	m/z 654.100	Digoxin-d3
10.07 10.07	m/z 103.070 m/z 194.160	N-Butylscopolamine
12.94 12.94	m/z 330.300 m/z 375.380	Nerifolin
11.52 11.52	m/z 94.080 m/z 152.210	N-Methylscopolamine
0.00	m/z 373.290 m/z 433.290	Oleandrin
10.39	m/z 403.100	Ouabin
12.94 12.79	m/z 82.050 m/z 138.070	Ricinine
4.53 4.53	m/z 138.200 m/z 156.130	Scopolamine

5.2.3.Calotropis:

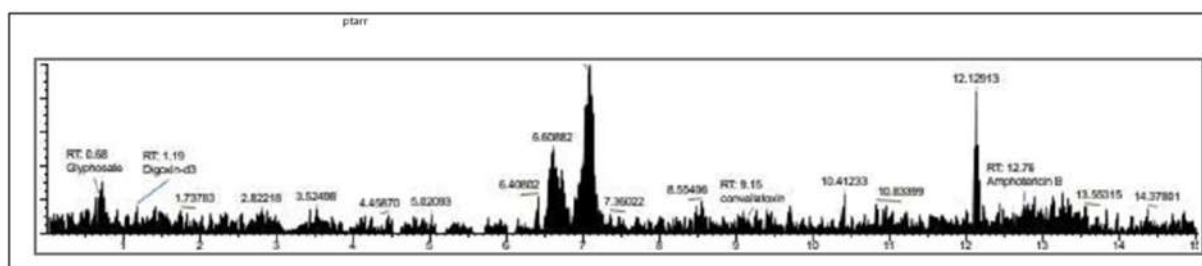
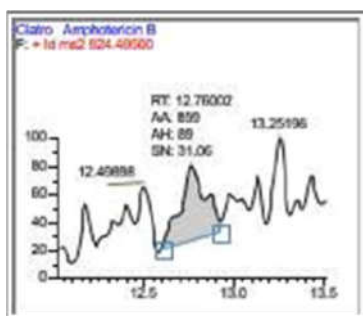


Fig 5.2.3 LC Chromatogram of Calotropis:
(Source: Self- Analysed)

Analysis of LC Chromatogram of Calotropis

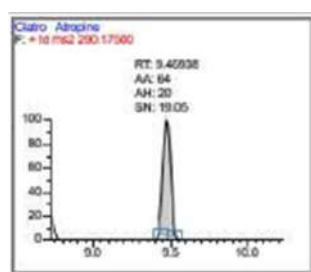
There are significant peaks at varying retention times, and some compounds have been identified using their RT values. (Fig 7.7) Dap-d3 at RT 1.19 minutes, indicating the strong elution of the said compounds off the column. There is a significant peak at RT 6.60882 minutes, indicative of the major compound in the sample. The other peaks identified are (Fig 7.4) convallatoxin RT 9.15 minutes and (7.1) Amphotericin B RT 12.76 minutes, There are a few other small, non-labeled peaks on the chromatogram as an indication of the sample complexity and presence of minor components or possible impurities.

5.2.3.1 Amphotericin B



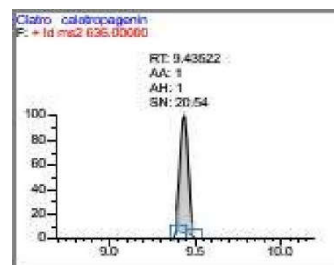
(Source: Self- Analysed)

5.2.3.2 Atropine



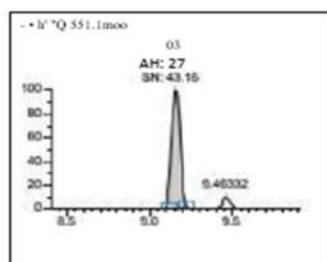
(Source: Self- Analysed)

5.2.3.3 Calotropagenin



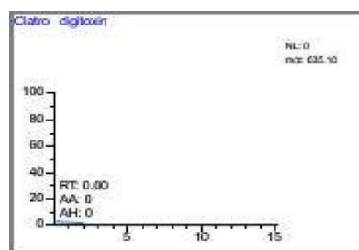
(Source: Self- Analysed)

5.2.3.4 convallatoxin



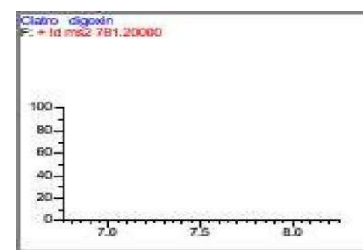
(Source: Self- Analysed)

5.2.3.5 Digitoxin



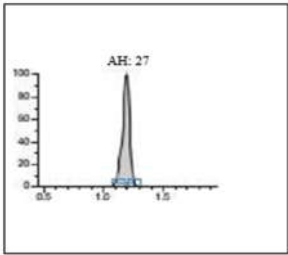
(Source: Self- Analysed)

5.2.3.6 Digoxin



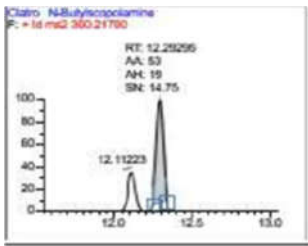
(Source: Self- Analysed)

5.2.3.7 Digoxin-d3



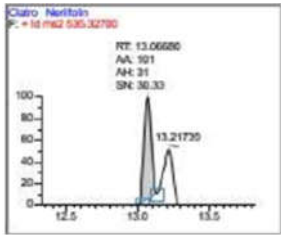
(Source: Self- Analysed)

5.2.3.8 N-Butylscopolamine



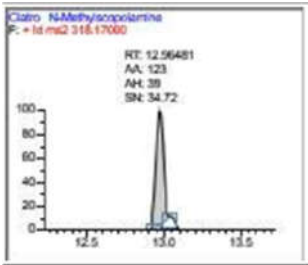
(Source: Self- Analysed)

5.2.3.9 Neriifolin



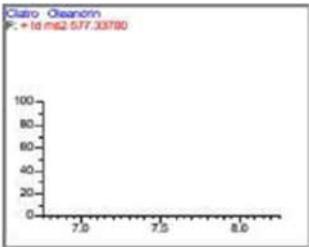
(Source: Self- Analysed)

5.2.3.10 N-Methylscopolamine



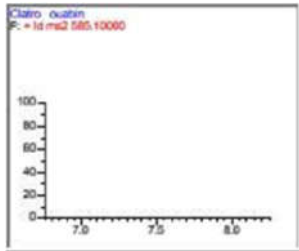
(Source: Self- Analysed)

5.2.3.11 Oleandrin



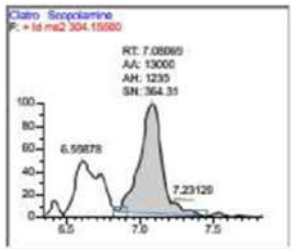
(Source: Self- Analysed)

5.2.3.12 Ouabin



(Source: Self- Analysed)

5.2.3.13 Scopolamine



(Source: Self- Analysed)

Table 5.2.3.14 Analytical Results: Compound Identification by RT and m/z

Retention Time (Min)	ION	Detected Compound
12.76 12.71	m/z 355.110 m/z 429.130	Amphotercin B
9.47 9.47	m/z 93.070 m/z 124.130	Atropine
0.44	m/z 206.000	Calotropagenin
9.15	m/z 369.200	Convallatoxin
0.00	m/z 635.100	Digitoxin
0.00	m/z 651.100	Digoxin
1.19	m/z 654.100	Digoxin-d3
12.29 12.29	m/z 103.070 m/z 194.160	N-Butylscopolamine
13.07 13.07	m/z 339.300 m/z 375.380	Nerifolin
12.96 12.96	m/z 94.080 m/z 152.210	N-Methylscopolamine
0.00	m/z 373.290 m/z 433.290	Oleandrin
0.00	m/z 403.100	Ouabin
0.00	m/z 82.050 m/z 138.070	Ricinine
7.08 7.09	m/z 138.200 m/z 156.130	Scopalamine

5.2.4. Nerium Oleander

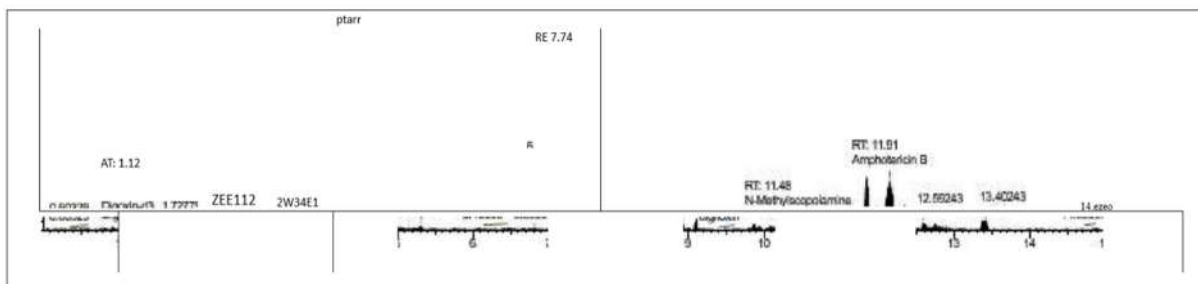
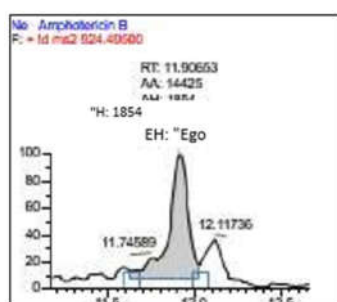


Fig 5.2.4 shows LC Chromatogram of Nerium Oleander
(Source: Self-d Analysed)

Analysis of LC Chromatogram of Nerium Oleander

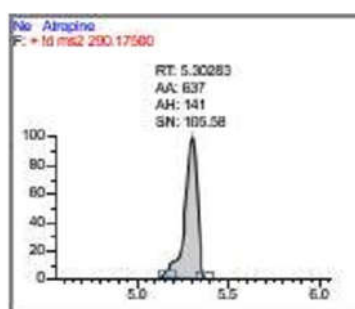
Prominent peaks are observed at varying retention times, with some compounds being given names based on their RT values. A peak at RT 1.12 minutes, for instance, corresponds to an early-eluting compound, whereas others at RT 11.48 and RT 11.91 minutes are labeled N-Methyl scopolamine (Fig8.10) and (Fig 8.1) Amphotericin B, respectively, indicating the presence of these pharmacologically active compounds in the sample. There are other minor peaks at RT 12.59043 and RT 13.42043 minutes, corresponding to other constituents or impurities. The clear resolution of such peaks shows how effectively the chromatographic method has performed to separate complex mixtures

5.2.4.1 Amphotericin B



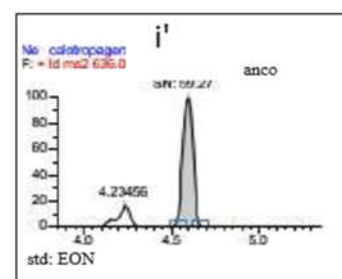
(Source: Self- Analysed)

5.2.4.2 Atropine



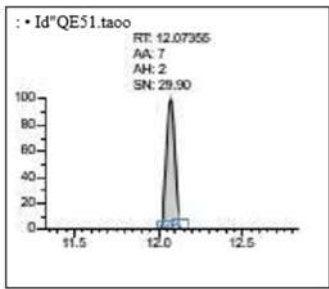
(Source: Self- Analysed)

5.2.4.3 calotropagenin



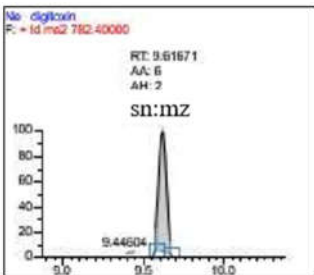
(Source: Self- Analysed)

5.2.4.4 convallatoxin



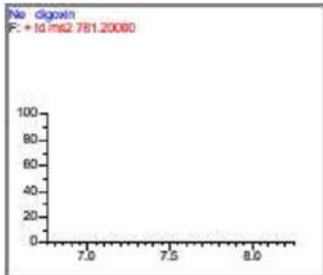
(Source: Self- Analysed)

5.2.4.5 digitoxin



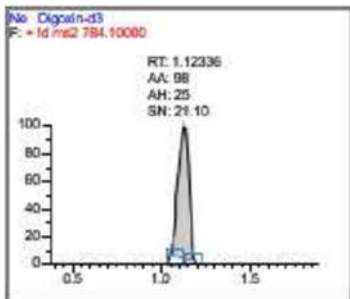
(Source: Self- Analysed)

5.2.4.6 digoxin



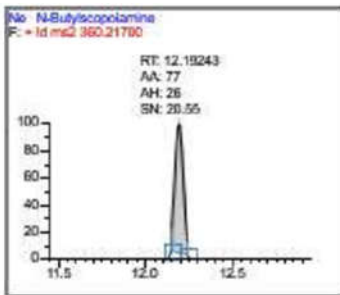
(Source: Self- Analysed)

5.2.4.7 Digoxin-d3



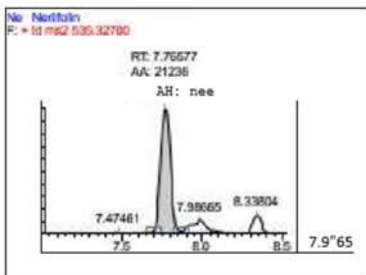
(Source: Self- Analysed)

5.2.4.8 N-Butylscopolamine



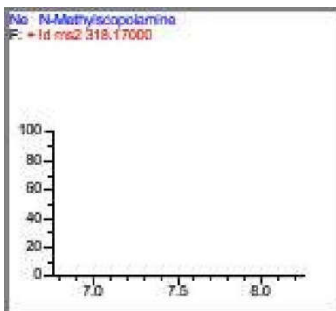
(Source: Self- Analysed)

5.2.4.9 Neriifolin



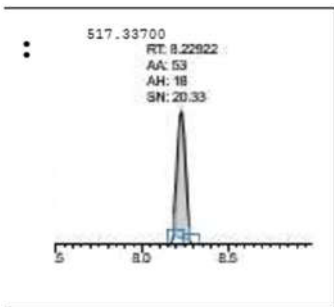
(Source: Self- Analysed)

5.2.4.10 N-Methylscopolamine



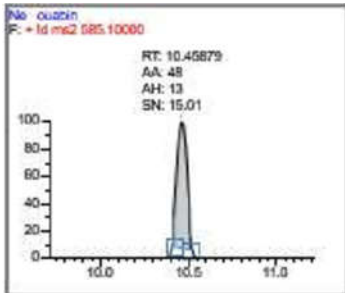
(Source: Self- Analysed)

5.2.4.11 Oleandrin

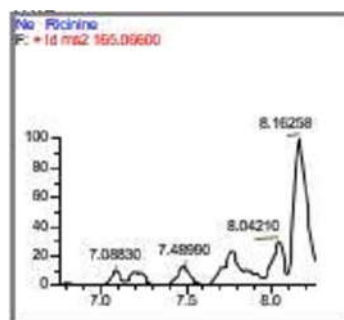


(Source: Self- Analysed)

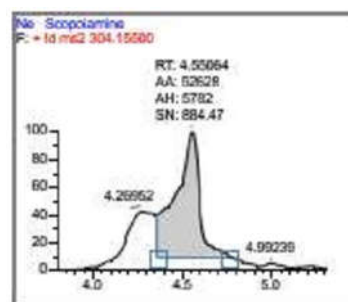
5.2.4.12 Ouabin



(Source: Self- Analysed)

5.2.4.13 Ricinine

(Source: Self- Analysed)

5.2.4.14 Scopolamine

(Source: Self- Analysed)

Table 5.2.4.15 Analytical Results: Compound Identification by RT and m/z

Retention Time (Min)	ION	Detected Compound
11.91 11.92	m/z 355.110 m/z 429.130	Amphotercin B
5.30 5.29	m/z 93.070 m/z 124.130	Atropine
4.60	m/z 206.000	Calotropagenin
12.07	m/z 369.200	Convallatoxin
9.62	m/z 635.100	Digitoxin
0.00	m/z 651.100	Digoxin
1.12	m/z 654.100	Digoxin-d3
12.19 12.19	m/z 103.070 m/z 194.160	N-Butylscopolamine
7.77 7.77	m/z 339.300 m/z 375.380	Nerifolin
11.48 11.48	m/z 94.080 m/z 152.210	N-Methylscopolamine
8.23 8.23	m/z 373.290 m/z 433.290	Oleandrin
10.46	m/z 403.100	Ouabin
0.00	m/z 82.050 m/z 138.070	Ricinine
4.55 4.56	m/z 138.200 m/z 156.130	Scopolamine

5.2.5. Seme carpus Anacardium

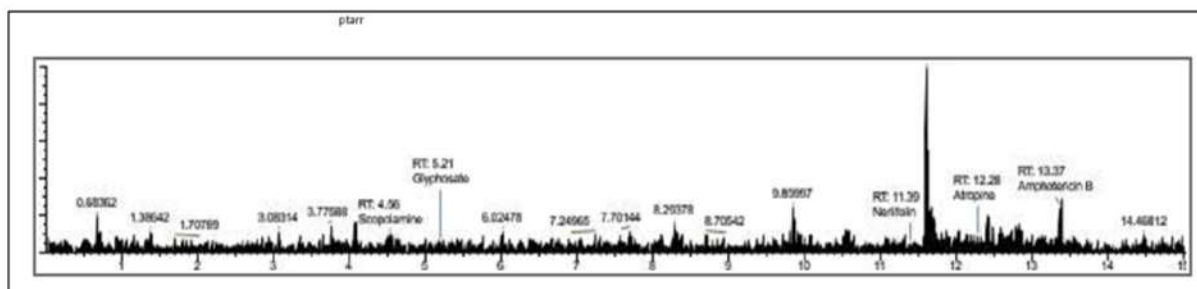
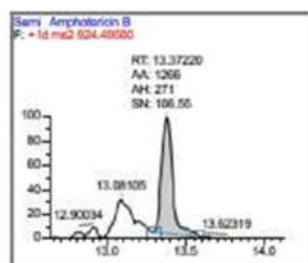


Fig 5.2.5. Shows LC Chromatogram of Seme carpus Anacardium
(Source: Self- Analysed)

Analysis of LC Chromatogram of Seme Carpus Anacardium

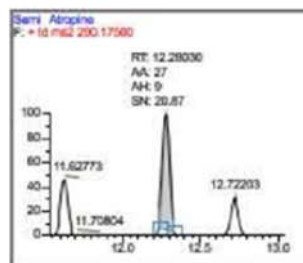
The chromatogram detects a number of major compounds:, (Fig 9.14)scopolamine RT 4.46 min, naphthalene RT 11.39 min, (Fig 9.2) atropine RT 12.28 min, and (Fig 9.1)amphotericin B RT 13.37 min. The highest peak is naphthalene, exposing it as the most prevalent compound detected using the conditions of analysis.

5.2.5.1 Amphotericin B



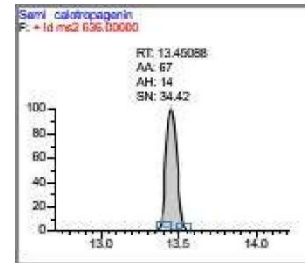
(Source: Self- Analysed)

5.2.5.2 Atropine



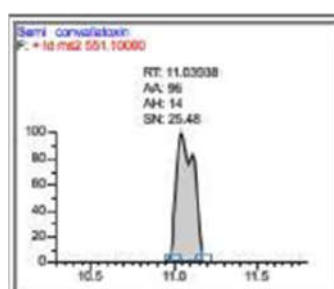
(Source: Self- Analysed)

5.2.5.3 Calotropagenin



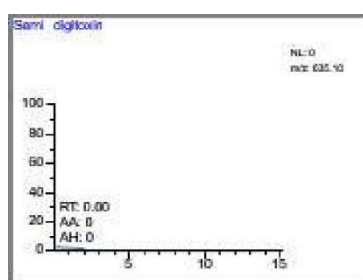
(Source: Self- Analysed)

5.2.5.4 Convallatoxin



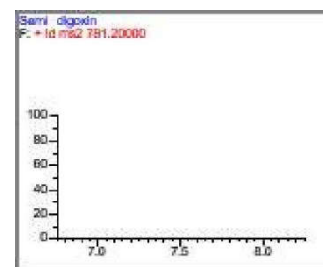
(Source: Self- Analysed)

5.2.5.5 Digitoxin

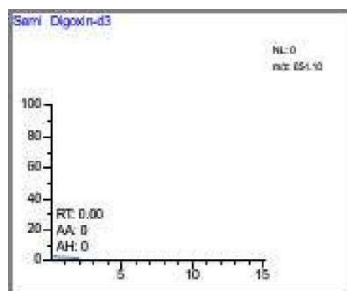


(Source: Self- Analysed)

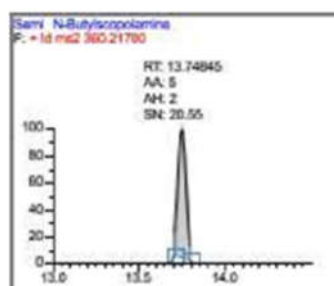
5.2.5.6 Digoxin



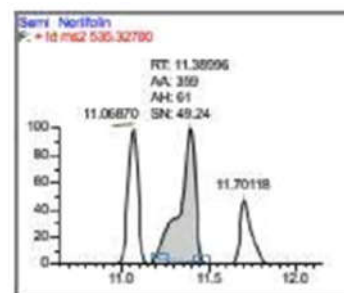
(Source: Self- Analysed)

5.2.5.7 Digoxin-d3

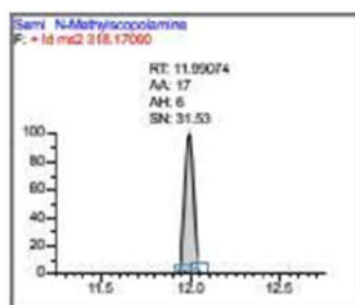
(Source: Self- Analysed)

5.2.5.8 N-Butylscopolamine

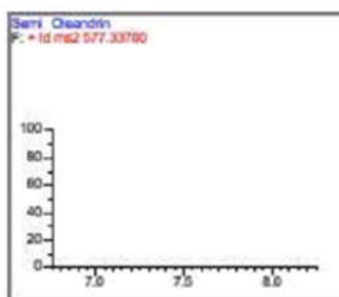
(Source: Self- Analysed)

5.2.5.9 Neriifolin

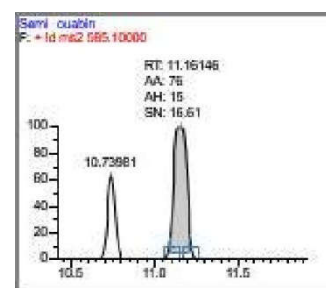
(Source: Self- Analysed)

5.2.5.10 N-Methylscopolamine

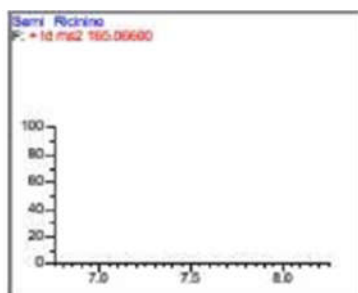
(Source: Self- Analysed)

5.2.5.11 Oleandrin

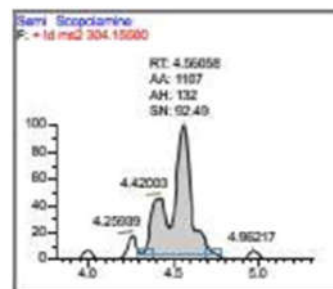
(Source: Self- Analysed)

5.2.5.12 Ouabin

(Source: Self- Analysed)

5.2.5.13 Ricinine

(Source: Self- Analysed)

5.2.5.14 Scopolamine

(Source: Self- Analysed)

Table 5.2.5.15 Analytical Results : Compound Identification by RT and m/z

Retention Time (Min)	ION	Detected Compound
13.37 13.37	m/z 355.110 m/z 429.130	Amphotercin B
12.28 12.28	m/z 93.070 m/z 124.130	Atropine
13.45	m/z 206.000	Calotropagenin
11.04	m/z 369.200	Convallatoxin
0.00	m/z 635.100	Digitoxin
0.00	m/z 651.100	Digoxin
0.00	m/z 654.100	Digoxin-d3
13.75 13.75	m/z 103.070 m/z 194.160	N-Butylscopolamine
11.39 11.39	m/z 339.300 m/z 375.380	Nerifolin
11.99 11.99	m/z 94.080 m/z 152.210	N-Methylscopolamine
0.00	m/z 373.29 m/z 433.290	Oleandrin
11.16	m/z 403.100	Ouabin
0.00	m/z 82.050 m/z 138.050	Ricinine
4.56 4.56	m/z 138.200 m/z 158.130	Scopalamine

Discussion:

The chromatographic data shown herein are representative of a mixture composition of an assortment of compounds in the samples under analysis, as evidenced by identification of many different peaks at multiple retention times (RTs). Each peak corresponds to a distinct compound, and several have been identified successfully by comparison of their RTs with those of known standard compounds. Interestingly, constituents with pharmacological activities such as nerifolin (RT 7.76 min), calotropagenin (RTs 10.19 and 10.61 min), ouabain (RT 11.14 min), amphotericin B (RTs 11.91, 12.76, and 13.37 min), atropine (RTs 5.27 and 12.28 min), convallatoxin (RT 9.15 min), N-methylscopolamine (RT 11.48 min), scopolamine (RT 4.46 min), naphthalene (RT 11.39 min), and Dap-d3 (RT 1.19 min) are found, showing that there exist therapeutic products and potential impurities. Reproducibility and consistency of the chromatographic process used is reflected by the repeated detection of some compounds, such as amphotericin B and atropine, at the same RTs in different chromatograms.

Besides these major peaks, various small and unlabeled peaks were observed at lower and higher RTs and can be markers for trace constituents, impurities, or unknown compounds, thus reflecting the sample complexity. Maximum resolution and sharpness throughout the chromatograms tell us a great deal about the effectiveness of the separation process, the result of which is that the method parameters—e.g., column selection and mobile phase composition—must have been optimal. Dominating peak identification, e.g., naphthalene, indicates dominance by some of the analytes in the sample, while early-eluting peaks such as Dap-d3 indicate more polar analytes. The presence of pharmacologically active compounds suggests the biological or pharmaceutical relevance of the samples, while the minor peaks suggest that there could be perhaps other components worth exploring, perhaps with alternative methods of analysis like mass spectrometry, in order to properly identify all the components. In general, the chromatographic analysis confirms both high analytical technique performance and sample matrix complexity and is a good basis for qualitative as well as possible quantitative examinations.

Chapter VI

Conclusion and Scope for Future Studies

Chapter VI: Conclusion and Scope for Future Studies

6.1 Conclusion:

In Conclusion, The Chromatogram of DATURA sample, using 1.00 mL injection in high-performance autosampler conditions exhibited good reproducibility. Major components were 3-hydroxy-propanoic acid hydrazide (20.28%), Tridecane (10.22%), and major fatty acid esters like hexadecanoic acid methyl ester (16.30%), 9,11-octadecadienoic acid (E,E) methyl ester (15.34%), 8,11,14-docosatrienoic acid methyl ester (12.89%), and methyl stearate (11.64%). They propose that the blend is a complex, complex blend of hydrocarbon and lipid-derived compounds with promise of a range of biological activity and worthy of further pharmacologic exploration. The constituents of the analysis showed Bhilawanol A to be the major compound at 93.22% of the sample, with significant minor constituents as Ginkgol (TMS) at 6.02%, and phenolic derivatives like 3-((4Z,7Z)-Heptadeca-4,7-dien-1-yl), and (Z)-3-(pentadec-8-en-1-yl). This represents a chemical composition ruled by Bhilawanol A as the major compound and backed by phenolic compounds most likely responsible for its bioactivities.

The chromatographic analysis of the sample was significantly of high chemical order of complexity by taking advantage of the fact that numerous well-resolved peaks at various retention times were observed. Identification of the major pharmacologically active constituents such as nerifolin, calotropagenin, ouabain, amphotericin B, atropine, convallatoxin, N-methylscopolamine, scopolamine, naphthalene, and Dap-d3 was achieved by precise retention time correlation with the authentic standards and thus defining the selectivity and specificity of the process. Best resolution and sharpness are indications that process parameters, e.g., column, composition of mobile phase, and flow rate, were fully optimized to provide successful separation of analytes of similar structure in the matrix. Reproducibility of retention times of primary analytes in several chromatograms is also an indication of the stability and reliability of the method. Aside from the major components, the existence of several unidentified minor peaks indicates the existence of other trace components, possible impurities, or degradation products, which need other analytical methods—like mass spectrometry or nuclear magnetic resonance spectroscopy—for a complete determination of structure. Control by a single peak, like that of naphthalene, indicates extensive quantitative fluctuation in concentration of compounds in the sample. Generally, the findings are in favor of the feasibility of employing the chromatographic method for qualitative as well as potential quantitative identification of

complex drug or biological samples and lay the foundation for further target-based analysis for complete characterization of all involved constituents.

6.2. Scope for Future Work

- The potential for future research on the forensic identification and detection of toxic plants by the use of GC-MS and LC-MS/MS is vast and huge. As shown in the thesis, there is a dire need for the development of thorough and systematic mass spectral and chromatographic prints data bases for even more diversified plant toxins that would greatly improve the speed and effectiveness of forensic identification.
- Future research should target being more specific to streamline and perfect extraction and analytical methods for increased sensitivity and specificity, particularly identification of trace amounts of toxins in complex matrices of biological and environmental origins.
- Further improvement can also be achieved with the addition of newer analytical instruments, including high-performance mass spectrometry and high-throughput automated technology, in speeding up and making toxicology analysis more accurate.
- Interdisciplinary dialogue among forensic scientists, botanists, and analytical chemists will be critical in enabling proper plant species identification and correct interpretation of toxicological findings. Research also needs to go on as a means of producing detection for the new threats since new poisons and new toxins continue to arise.
- Lastly, forensic outcome correlation with clinical toxicology can optimize case interpretation of poisoning to further criminal investigation and public health intervention in the long

Chapter VII

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Chapter VII: References

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