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PHYTOTOXINS IN FORENSIC MEDICINE AND INVESTIGATIONS: AN OUTLOOK TOWARDS THE INCREASING RELEVANCE

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ABSTRACT

The phytochemicals derived from plants are used in forensic sciences and emerge as an innovative frontier which provides extraordinary insights into mysteries of nature using the cutting-edge technique of phytotoxin analyses. The work explores the connection between forensic science and phytochemistry which highlights the phytochemical details of toxic plant secondary metabolites that serve as a valuable marker for forensic investigations, as the plants contain bioactive substances more often benefiting to the health of individuals, but in some cases produce serious adverse effects. The review offers an in-depth understanding of phytochemicals in forensic investigations to detect these criminal offences including murder by poisoning and suicides. Toxins of plant-origin are secondary metabolites of natural origin which pose a threat to the health of humans and animals and the plant toxins play a major role in forensic investigations by permitting detailed elucidation and analyses of herbal toxins, and biotechnology and molecular tools can detect specific toxins, as well can detect specific poisonous, which uses a probe binding with specific receptors molecules. Forensic toxicology is a hybridization of contemporary analytical chemistry and fundamental toxicology and their implementation within the legal framework to answer questions that arise during judicial proceedings linked to intoxication. More researched are needed to deeply implemented to understand the significance of medicinal plants in forensic investigations to detect the criminal offenses. Additionally, to provides a deep understanding of chemical substances that can impact human life positively or negatively with different doses as well as identifying the optimal or overdose concentrations for either treatments or poisonous effects using recent biotechnological approaches. So, the present review summarises the major phytotoxins and their relevance in forensic medicine and investigations.

KEYWORDS: *. Forensic medicine, forensic pathology, forensic toxicology, phytotoxins*

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INTRODUCTION

The forensic phytochemistry is an emerging new-fangled discipline in the multifaceted are of forensic sciences that involves botany and chemicals uses [*Coyle, 2004*]. The forensic phytochemistry influences the plant-based evidence to discover or lighten the mysteries behind the investigation that generally avoid the old-style investigative methods [*Hall and Byrd, 2012*]. The forensic phytochemistry is most important over the traditional forensic methods owing to the connection between the nature and crime [*Bock and Norris, 2015*]. The investigation using phytochemical fingerprints arises as promising method for many unravelling the mysteries hidden that hidden in the toxic plants. Moreover, the traditional forensic methods primarily focus on human based evidences whereas, forensic phytochemistry uses the different chemical fingerprints which engraved on plant matter for investigation [*Coyle et al., 2005*]. This growing field was recognized since the mid-1900s, precisely during the investigation of the case of Charles Lindbergh baby-kidnapping, Arthur Koehler displayed the wood as evidence to the court setting [*Coyle et al., 2001*].

Since ancient times, the plant usage in forensic field at microscopic as well as macroscopic levels including their organs like stem, leaves, seeds, root and fruits, tissues materials such as spores, fibres, pollen grains and cork, and also, their secondary metabolites, DNA, starch grains and isotopes were used as forensic evidence [*Quideau et al., 2011; Kittakoop et al., 2014; Bozorgi et al., 2017; Awuchi, 2019*]. However, 400,000 plant species are approximately documented yearly from terrestrial and aquatic environment which contains autotrophic, eukaryotic and multicellular organisms [*Sharrock et al., 2014*]. Usually, the plant part carrying a silent witness to the surroundings including leaves, pollen grains and seeds [*Oliveira et al., 2023; Qu et al., 2021*]. Even though the plants are widely used in detoxification [*Jyoti et al., 2022*] some plants are highly poisonous and toxic [*Mrunali et al., 2022*], and the toxin identified in plants are secondary metabolites which act as a defence mechanism towards pathogens, other competing plants and herbivores [*Coyle et al., 2023*] and possess significant protecting role and important risk to human when they consumed or contact. Generally, the toxins are widely distributed in their chemical form which affecting health of humans. Many organic compounds like tannins, terpenes, alkaloids, flavonoids, cyanogenic and cardiac glycosides, proanthocyanidins, lignans, oxalates, nitrogen-containing compounds, phenylpropanoids, resins, amino acids and proteins [*Bernhoft et al., 2010*]. In addition, some poisonous plants are accumulating the inorganic compounds which detected in soil.

However, the plants are generally reported for health benefits but it has some side effects to humans [*WHO, 2013; Dar et al., 2017*]. Hence, the plants are not only recognized for their health benefits but it has been used for various adverse purposes including severe injury, death and antagonistic activity through controlling poisonous substances concentration. The plants that are poisonous are one of the natural poisonous materials to cause conflicts or sometimes death [P*illay and Sasidharan, 2019*]. For example, *Nerium Oleander* is one of the important medicinal plants, grown all the fields and appeared in two colours like white and pink that was used for skin treatment but it has some side effects due to its toxic nature of entire plant parts including seeds, leaves, stem and root that contain cardiac glycosides which cause cardiotoxicity. The chemistry of the plants delivers the complete understanding of chemical substances that are affecting human or animals in the positive or negative way. For example, the plant derived sedative namely opium from poppy plant is mainly used for pain relief [*Drewes et al., 2013*]. The opium contains epoxy morphinan ring in their structure like codeine, morphine, buprenorphine, oxycodone, hydrocodone and hydromorphone. Morphine was first isolated compound from plant and used for pain relief [*Drewes et al., 2013*]. In addition, the illegal use of opioids like heroin is more often nowadays. The studies highlighted the health risk of opioids when it exceeds its limits resulting diversion and exploitation [*Al-Tayyib et al., 2017*].

Therefore, the plants such as medicinal or toxic plants offers the solid indication during the forensic investigation in several criminal cases like theft and suicide. The research revealed that, the professional criminals choose the plant toxic materials owing to its cost effectiveness and availability [*Dubey et al., 2018*]. Accordingly, the poison

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concentration is also depended on the criminal's choice. Moreover, many ways are there to detect the criminal offenses and toxic substances using biotechnology and molecular tools through both qualitative and quantitative analyses [*Dubey et al., 2018*]. Hence, our review focuses how plants can be used in forensic investigation for criminal offence detection.

Plant poisoning historical cases: Apart from the theoretical application, the forensic phytochemistry created a important effect on demonstrating the real-world case studies which emphasizes the uses of plant material during criminal investigation [*Anywar, 2020; Dubey et al., 2018*]. The following explained cases brightly demonstrated the how the plant-based evidences are essential for solving crimes that provides the exclusive juncture among the forensic sciences and nature. The numerous cases are related to incidents of plant-poisoning that have been shown to understand the relevance of plant-based toxicology in forensic investigations.

Case study of poisonous garden: This high profiling case highlighted the forensic phytochemistry that played a major role in examination of victim's surroundings by the investigators [*Dubey et al., 2018*]. Consequently, the plant sample collected from victims surrounding exposed the toxic compounds presence from a sporadic plant species which indicated the exact geographical origin as well as poisonous agent identification leads the way for criminal arrest [*Raje et al., 2022*]. The criminals widely used the plant derived toxic materials during theft, rape case and homicide. The toxicity can be manifested in various ways such as direct contact resulting skin irritation, internal poisoning and inhalation.

Case study of Socrates' death: This case highlighted the deliberate usage of plant derived toxin for processing the death. Here, the Greek philosopher Socrates was died due to the consumption of a cup of hemlock (*C. Maculatum*) which contained coniine - a powerful neurotoxin resulting respiratory paralysis followed by death [*Hotti and Rischer, 2017*].

The Borgia family poisonings case: The infamous Borgia family was active when the Italian Renaissance and also, accused for having the toxic plants namely *Atropa belladonna* (Nightshade) for political assassinations that plant contains alkaloid atropine which highlighted the use of toxin from plants for political conspiracy [*Retief and Cilliers, 2000*].

Case study of locoweed livestock poisoning: In this case, forensic investigation explored the plant derived toxin causes death in livestock in the western regions of North America. The *Astragalus* and *Oxytropis* plants are well known poisoning agent in cattle form. The locoweeds consumption by horses and cattle leads to death due to locoism, a neurological condition [*Stegelmeier et al., 1999*].

Water Hemlock poisonings case: This case proved the accidental ingestion of *Cicuta* species (Water hemlock), is most dangerous toxic plant in North America by Meriwether Lewis and Christopher mccandless resulting poisoning leads death which was identified during forensic investigation [*Dodson nd Dunmire, 2007*].

Foxglove poisonings case: Generally, digitalis compounds are well known for medical uses but inappropriate or overuse of such compounds are dangerous. The accidental or intentional ingestion of large quantity of *Digitalis purpurea* (foxglove) cause death due to cardiac glycosides which can be detected during forensic analysis [*Norn and Kruse, 2004*].

Case study of cassava cyanide poisoning: During the forensic investigation, *Manihot esculenta* (Cassava) is reported for the cyanogenic glycosides that can able to release hydrogen cyanide during metabolization. The cyanide poisoning has occurred in the region wherein cassava is the main dietary source. The cyanide was detected from improperly processed or consumed inappropriate use of cassava products in post-mortem samples [*Siritunga and Sayre, 2004*]. The historical cases are underlined the serious relevance of herb-associated toxicology in forensic analyses and also, particular plant-derived toxin identification, mode of action also important to distinguish the accidental and intentional poisonings during forensic analysis. To enhance the forensic toxicology field, the advanced analytical techniques have to be improved for detection and quantification of herb-derived toxins.

Toxic plants in forensic pathology: In forensic pathology, plant derived toxin played a major role in finalising the death cause. In addition, the toxic plants make multiple challenges ranging from ac-

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cidental poisonings to intentional or homicide in forensic pathology. During the investigation, the forensic pathologists have collaborated with toxicologists and Phyto chemists for identification and better implication of toxin from plants during postmortem examinations. The complete investigations and thorough documentations are important steps to discover the toxic plant role in the cause of death. When the forensic professionals deal with the toxic plants in forensic pathology, many considerations and suggestions have to be followed.

Accidental poisoning: The accidental poisoning occurred when toxic plants were ingested by children or adult unknowingly or without knowing their toxic effect resulting poisoning. The Forensic pathologists have to examine the opportunity of accidental ingestion during the cause of death determination [*Strzelecki et al., 2010*].

Suicidal poisoning: The plant derived toxin act as suicide material when someone **is** deliberately ingesting the toxic plant as suicide method and to discover the plant toxin during postmortem examinations required detailed questioning about intake of plant material [*Strzelecki et al., 2010*].

Homicidal poisoning: In some cases, the toxic plants used as homicide material and the investigation is needed for pattern identification that propose the specific plant toxins presence [*Sinha et al., 2017*].

Identification of plant toxins: The identification of plant toxin in samples of postmortem analyses is very complicated owing to various circumstances that includes toxin degradation, postmortem changes and the requirement of dedicated analytical techniques. For better identification, the collaboration of Forensic pathologists and forensic toxicologists are needed for quantification and identification of toxin in the particular samples [*Shedge et al., 2019*].

Postmortem changes and decomposition process: The changes during postmortem and decomposition of body are affecting the detection of herbal toxins. Hence, postmortem analyses are recommended to be performed earlier to reduce the complications and to get the results with more precision [*Almulhim and Menezes, 2020*].

Clinical presentation vs. Autopsy findings: If inconsistencies are occurred in poisoning by a plant, the chances for clinical symptoms are there, which will be reported prior to death and the postmortem analyses which emphasize the significance of complete toxicological investigations are needed to confirm the presence of plant toxins [*Nelson et al., 2007*].

Histopathological examination: Plant toxins are able to cause exact histopathological changes in organs resulting organ damage which provide the additional knowledge about the plant toxins effect on tissues and also contributing the cause of death determination during histopathological examination [*Kuete, 2014*].

Documentation and case history: The comprehensive case documentation is maintained by forensic pathologists for accurate documentation in the death surrounding, and the information connected to the exposure or intake or to toxic plants [*Dolinak et al., 2005*].

Toxic principles of plant origin: Various plants contain a number of phytochemicals including saponins, flavonoids, phytosterols, steroids, tannins, catechins, alkaloids, triterpenes, flavanols, pentacyclic triterpenoids, reducing sugars, xanthones and flavanones [*Ayman et al., 2023; Gobika et al., 2024; Gobianand et al., 2024; Imran et al., 2024;*].

In forensic science, the plant toxins were studied for identification, analysis, and interpretation of toxic substances obtained from plant origin, specially related to criminal or legal investigation [*Patil et al., 2023*]. The plant toxin plays a chief role in the forensic case which associated with environmental crimes, biological warfare and poisoning. It is already known that; some phytochemicals are harmless and has health benefits. Consequently, the plant toxin in forensics were categorized into different classes includes alkaloids, are naturally occurring organic compound which consist of nitrogen along with various structural forms [*Li et al., 2021*], glycosides, a sugar molecule related to a non-sugar moiety like cyanogenic glycosides which is present in cassava that creates significant toxicity [*Refahy, 2011*]. Similarly, proteins from plants like Mucunain and Toxalbumin, act as toxins and cause cell damage leads organ damage [*Dickers et al., 2003*] and also, Lectins and Protease inhibitors are interfered with protein synthesis resulting toxicity and nutrient deficiency [*Kim et al., 2009*] and also, phenols, a secondary metabolite from plant act as defence material and

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causes lower protein digestibility [*Toschi et al., 2022*], tannins, a phenolic compound act as plant toxin and causes death [*Maugeri et al., 2022*], anthraquinones like Hypericin primarily detected in *Hypericum perforatum* and damage the proteins, lipids, and DNA, resulting in o oxidative stress and cell death [*Shukla et al., 2017; Abegg et al., 2024*], Sesquiterpenes, picrotoxin is derived from the *Anamirta cocculus* plant seed and disturbed the normal neuronal signalling leads high neuronal excitability and potential seizures [*Pressly et al., 2020*], cannabinoids, present in *Cannabis sativa* acting as plant toxin [*Alves et al., 2020;* Śmiarowska *et al., 2022*].

Plant toxin detection: The elucidation and quantification of herbal toxin compounds can be achieved by phytochemical fingerprinting that played a crucial role in the forensics. This method provides precise and sensitive measurements which helps forensic investigators in identifying the particular toxin and to quantify its concentration. Mainly, two important techniques are employed in the analyses of plant toxins and they are chromatography which includes gas chromatography [*Mukadam et al., 2021*] and liquid chromatography [*Fanali et al., 2017*] and mass spectrometry which includes gas chromatography-mass spectrometry (GC–MS) [*Ahuja et al., 2023*], liquid chromatography-mass spectrometry (LC–MS) [*Al-Rubaye et al., 2017*], nuclear magnetic resonance (NMR) spectroscopy [*Alkefai et al., 2022*] and enzyme-linked immunosorbent assay (ELISA) [*Sakamoto et al., 2018*]. These analytical techniques provide the complete method for detection and quantification of plant toxin during the forensic investigations and also, the advancement in sensitivity, instrumentation as well as data analysis are required to improve and consistency of these techniques.

Challenges in identification of plant toxins: During postmortem analyses, the plant toxin identification is a multifaceted process due to many challenges that include metabolic alterations due to degradation. Thus, the factors require careful consideration for accurate toxicological assessments in cases involving potential toxication by plant materials. Forensic analysts need to understand these challenges to provide accurate and reliable results which including rapid postmortem changes [*Forbes et al., 2017*], metabolism [*Szeremeta et al., 2021*], Biotransformation by microorganisms [*Der Rogowska-van Molen et al., 2023*], Toxin degradation [*Uekusa et al., 2015; Kot-Wasik, 2004*] and Forensic context [*Yu et al., 2021*].

Conclusion

Plant-derived toxins are natural secondary metabolites which creates important risks to human and animal health and the plant toxins played a major role in forensic investigations by permitting detailed identification and analysis of plant toxins. For sample analysis, various advanced chromatographic and spectroscopic techniques are used for detection and quantification of plant toxin. Based on the analysis of plant toxin profiles, the forensics offer clear vision on crime scenes, elimination or identification of plant toxins. Overall, the forensic phytochemistry improve the accuracy and reliability during criminal investigations.

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