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ORIGINAL ARTICLE

Analysis and Protein Profiling of Toxic Plants and their Relevance in Forensics

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ABSTRACT

Plant toxins and poisons are deadly weapons which can kill humans without any bloodshed or violence. These deadly plant weapons in the form of extracts or plant tissue as whole are used by culprits for burglary, rape, and murder. They are also used in many suicide cases. In India, numerous murders, unsolved deaths and crimes have been related to the use of plant toxins. Criminals use them often as they are freely available at virtually no cost. The plant extracts or plant tissues can often be found on the crime site or even on the murderer or suspect. In this project, research has been initiated to study the polymorphism of proteins found in the different poisonous plants in India and identify its forensic importance. The profiling or polymorphism has been done using SDS-Page technique, which shows a series of Protein Bands present in the plant tissue used. This data shows the typical proteins present in the different plants and the polymorphism of plant proteins can be used as a Protein Fingerprinting to identify the toxic plant used to cause harm, death, murder on a crime site. The Protein fingerprint thus obtained can be used as an evidence to identify a particular death with the specific symptoms as well as the toxic plant used in the crime.

KEYWORDS | plant poison, SDS-PAGE, protein profiling, plant toxins, proteins

INTRODUCTION

A few plant species that are poisonous or harmful to humans are commonly found in our gardens or planted as roadside trees. Plant poisons are deadly weapons capable of taking down one's life in dark and mysterious ways. They have a unique appeal for criminal minds. Plant poison is used as a weapon or "Botanical Weapon" (BW) by the criminals in thefts, robbery, and murder.[4]

Poisoning can take many forms, including contact (skin irritation)[2], absorption (causing internal organ poisoning), retention (by the dermal upper layer), and aerosolization (by the respiratory system).[5,9] Castor bean seeds (Ricinus communis) and Jequiriti beans (abrusprecatorius), for example, have been known for their toxicity since antiquity. One of the poisonous plants is the oleander, which can cause poisonous effect while cooking of food.

Botanical toxins may be a product of toxic and poisonous plants, or they may be a harmful component of plants.

The most important poisonous concepts found in plants are alkaloids and other common components. Proanthocyanidins, diterpenes, flavonoids, tannins, cardiac and cyanogenic glycosides, cardiac and cyanogenic

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glycosides, phenyl propanoids, lignans, nitrogen-compounds, gums, oxalates, and certain amino acids or proteins. These cyanogenic glucosides are silent killers in a toxic-crime because they are easily available. They have played a very important role in crimes and romance. Research was initiated to study the polymorphism of proteins found in the different poisonous plants in India. This study would help in identifying the plant tissue extract found at the crime site where death occurs due to ingestion of poisonous plant.

MATERIALS AND METHODS

Plant Material collection: Leaves and seeds of different toxic plants commonly found on roadsides were collected for the study. The five plant samples were: *Dhatura stramonium, Parthenium hysterophorus, Riccinuscommunis, Calotropis* and *Nerium Oleander.*

Preparation of Extracts of wet and dry plant samples: The fresh and dry leaves of the plants were ground in a Protein Extraction Buffer (PEB) (10 mL: 0.625 mL of 1M Tris-HCl, pH6.8 + 2 mL 10% SDS (w/v) + 1 mL 1M DTT (Dithiothreitol) + 1 mL 100% Glycerol + 5.375 mL distilled water) and were incubated for 24 hours. These extracts were then centrifuged for 10 minutes at 10,000 rpm and the supernatant stored at 4°C for all experiments.

Quantitative Analysis of Protein in Plant samples by Biuret method:

Biuret or Alkaline Copper Reagent: 50 mL 2% Na2CO3 in 0.1% NaOH + 0.5 mL 2% sodium-potassium tartrate + 0.5 mL 1% copper sulphate.

Standard Protein Sample: Bovine serum albumin (BSA) solution- 5mg/mL.

Methodology: Distilled water was added to the unique algorithmic of standard protein solutions and unknown samples, resulting in a final volume of 1.0 mL. 5.0 mL. Biuret reagent was added to both tubes, blended thoroughly and kept for 10 minutes at room temperature. Each tube's optical density was measured at 520nm. The Calibration graph was created based on the readings.[3]

Isolation and Characterization of Proteins by SDS-PAGE

SDS-PAGE (Laemmli, 1970) designed a method for isolating and characterization of proteins. In an Eppendorf micro centrifuge tube, 20L of protein sample extracted from the plant samples (both fresh and dried samples) and 10L of (1X) loading dye were combined (1.5 mL). The sample was warmed at 100°C for 2-10 minutes before being centrifuged for 1 minute. Using a micro pipette, the sample was placed in the gel wells. The gel should be run at a constant voltage (100V) until the tracking color reaches the bottom. Using coomassie brilliant blue R-250, the gel was stained with staining pigment. Kept that gel in the staining for one night. After overnight staining, the solution was poured out and the gel rinsed several times with water. Approximately 50 ml of destain solution was added to the container and kept overnight. The gel was eventually exposed against a white background.

DISCUSSIONS AND RESULTS

The present study deals with the protein profiling or fingerprinting of the commonly available toxic plant leaves. Number of plants are highly toxic to humans and animals if ingested or rubbed on the skin. These plant extracts are used by criminals to cause death or harm to their victims. Since these plants are freely available and can be obtained in large quantity these are used by small-time or first time criminals.

The total protein once extracted was quantified by Biuret method. The estimation of total soluble protein in the plant samples was estimated by using the standard.

Sl.No	Samples	Protein Concentration (mg/ml)
1.	Oleander fresh	1.15
2.	Oleander dry	1.76
3.	Calotropis fresh	0.76
4.	Calotropis dry	1.0
5.	Riccinus fresh	1.23
6.	Riccinus dry	1.57
7.	Datura fresh	0.5
8.	Datura dry	1.96
9.	Parthenium fresh	1.08
10.	Parthenium dry	1.42

Table 3 The total soluble protein extracted is higher in dry samples, since the amount of fresh plant tissue being weighed would be lesser as compared to dry tissue.

Nerium Oleander Plant

The fresh and dry leaf extracts show a different protein banding pattern (Table :1).

The presence of lower M.wt protein bands in dry tissue could be due to denaturation & breakdown of proteins found in the fresh tissue.

Protein bands Obtained	Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
1	12.6	8.9
2	10.0	7.07

Table 1 Molecular Weights of Protein bands Obtained in Oleander leaves

Calotropis Plant

The fresh and dry leaf extracts show a different protein banding pattern (Table: 2). The pattern of peptide bands obtained in Calotropis leaves show many similar bands having the same Molecular Weight. Thus, it can be assumed that due to the condition of the leaf sample the proteins may vary in Molecular weight when further denatured using SDS- PAGE.

Riccinus Communis

The fresh and dry leaf extracts show a similar protein banding pattern (Table:3). In Riccinus also similar protein bands are obtained in fresh leaves and dry leaves. The Ricin protein toxin which is mostly produced in the seeds of Riccinus plant has a molecular wt. of 65 Kda. In the fresh leaf extract we find a band for 65 kDa which could be the Ricin protein. But in the dry leaf extract it has broken down into its subunits and peptides.

Protein bands Obtained	Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
	210	7943.2
	121	210
	125.9	100
	67	25
	25	20
	14	14
	7.07	7.07
	5.01	5.01

Table 2 Molecular Weights of Protein bands obtained in Colotropis leaves

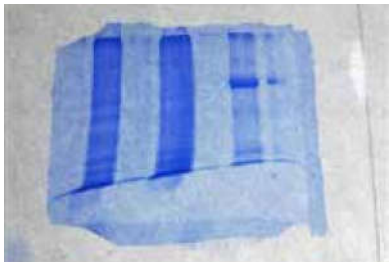

Figure 1 Calotropis protein bands On SDS PAGE gel.

Protein bands Obtained from Riccinus Communis

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
7943	2511.8
2511.8	316
562	112
65	39.8
12.6	20
8.9	12
6.08	5.6
8.9	12
6.08	5.6
4.47	5.01
3.5	-

Table 3 Molecular Weights of Protein bands obtained in Riccinus Communis

Datura

The fresh and dry leaf extracts of Datura leaves show a very similar protein banding pattern (Table: 4). Here we observe that almost all the bands obtained in the both fresh and dry leaf extracts are of similar Molecular weights. Thus, here we can clearly develop a specific fingerprint pattern of SDS-PAGE separated protein bands in order to identify Datura plant extracts found in crime site.

Parthenium

The fresh and dry leaf extracts show a different protein banding pattern (Table: 5). The presence of lower M.wt protein bands in dry tissue could be due to denaturation and breakdown of proteins found in the fresh tissue.

Protein bands Obtained from Dhatura

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
15848	3981
210	121
121	100
100	50
50	48
48	11.2
11.6	9.5
9.5	8.9
8.9	5
6	3.7
3.9	–

Table 4 Molecular Weights of Protein bands obtained in Datura

Protein bands Obtained from Parthenium

Fresh Tissue Protein MW [kDa]	Dry Tissue Protein MW [kDa]
7943	3981
1584	210
210	100
100	67.6
67.6	20
10	10
5.6	3.16
3.9	–
3.16	–

Table 5 Molecular Weights of Protein bands obtained from Parthenium

This study would thus help in identifying the plant tissue extracts found at the crime site where poisoning and death has occurred due to ingestion of poisonous plants.

In the present study, we find that many of the protein bands in wet and dry tissue are same except for some bands which are absent. Thus, a protein SDS profile would help in identifying the plant species.

Pandey *et al.*,[8] (2017)used SDS-PAGE to extract proteins ranging in size from 45 kilo Dalton (kDa) to roughly 15 kDa for Ricinus communis and 45 kDa to roughly 10 kDa for cannabis sativa, respectively. They discovered that both of the methods used such as UV spectrophotometry and SDS-PAGE, are indispensable in analysing plant proteins, and that the methods can be of great assistance to scientists and other labs concerned with plant and proteomics management.

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