Short Communication

Thin Layer Chromatographic Analysis of Some Commonly Used Poisonous Seeds

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ABSTRACT

Forensic scientists are often confronted with cases where seeds of poisonous plants are used in the commission of crime. Identification of these poisonous seeds is always a tough job because literature does not provide any significant reference work regarding this important aspect. In the present work, five seed samples of four different poisonous plant species were selected and Thin Layer Chromatography (TLC) was employed for the separation of their constituents. While advanced instrumental techniques are available, TLC is an economical and simple method that can be used in any laboratory. Various solvent systems were tried for analysis, and led to the discovery of an effective solvent system. This study is hoped to help forensic toxicologists in answering some queries related to TLC analysis of plant poisons.

Key Words: TLC, Thin layer chromatography, Poisonous seeds

Introduction

History reveals that poisoning due to plant seeds has been common from very early times, and knowledge of poisonous plants is perhaps as old as human civilization itself. In many countries where plant poisons are commonly available, people use them as a means to commit assault, homicide, or suicide. In India, seeds of *Datura fastuosa* are commonly used to stupefy people in facilitating robbery or assault. Seeds of *Abrus precatorius* have been used for homicidal purposes. Children are often involved in accidental cases. Swallowing of *Croton tiglium* seeds by mistake can produce fatal results. Plants often contain alkaloids, glycosides, terpenes, essential oils, acids, peptides, proteins, gums, resins or tannins as important active constituents, and these are usually concentrated in roots, seeds, leaves, or flowers.[1] Unfortunately, seeds of poisonous and nonpoisonous plants sometimes resemble each other. While, many poisonous plant seeds have unpleasant taste, some are tasteless or may even possess a pleasant taste, which can make recognition of poisonous varieties very difficult.

Perusal of avalable literature reveals that substantial efforts have been made to identify various physical and chemical characteristics of plant poisons, including thin layer chromatography (TLC) analysis of some plant poisons.[2,3] Some useful data related to TLC analysis of some plant alkaloids have been reported earlier.[4,5] A survey of literature reveals the utility of promising and advanced chromatographic techniques (HPLC and GC) for the detection of these poisons. LC-MS and Matrix Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF)-MS technique has been employed for the identification of *Ricin* from crude plant materials.[6] In one non-fatal case, serum and urine specimens were collected after five days and Liquid Chromatography-Electro

Spray-Mass Spectrometry (LC-ES-MS) was done to detect the presence of *Digitalis purpurea*.[7] Likewise, application of GC-MS technique for the quantization of tropane alkaloids in biological materials like serum and urine have been proposed.[8] The effects of chronic toxicity of *Aconitine* on electrocardiogram, and tissue con-

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centration of *Aconitine* and its metabolites in mice were observed using Gas Chromatography/Selected Ion Monitoring (GC/SIM).[9] An unusual case of poisoning by white seed variety of *Abrus precatorius* has been reported that caused serious manifestations in a middle-aged male. Poison traces were recovered after a prolonged duration of hospital treatment without any subsequent complications or sequelae. This case was reported on account of its rarity.[6] A simple High Pressure Thin Layer Chromatography (HPTLC) method was used for the quantification of gallic acid and ellagic acid from the seeds of *Abrus precatorius*, whole plant of *Phyllanthus maderaspatensis* and flowers of *Nymphaea alba*. The method was found to be simple, precise, specific, sensitive and very useful for the routine quality control of herbal raw materials.[10]

By observing the physical characteristics of plant material, a rough idea about the type of the poison can be made, but its precise identification and quantitation is a crucial and important factor in forensic toxicology cases. Sometimes, insufficiency of sample can be problematic, and prove to be a reason for the failure of effective analysis.

Materials and Methods

Seeds of *Cerbera thevetia, Ricinus communis, Croton tiglium,* and *Datura fastuosa* were crushed and dipped overnight in the following organic solvents: methyl alcohol, petroleum ether, acetone, and ethanol for the preparation of extracts. Using fine capillary tubes these extracts were spotted on a precoated TLC plate and allowed to dry for a few minutes. A TLC developing chamber containing the solvent system was properly saturated using filter paper strips, and the spotted TLC plate was placed vertically in it, and the chamber was covered with a lid. Separation of the samples was achieved after running the solvent system for a distance of 10 cm from the point of spotting. The TLC plate was then removed from the chamber and dried at room temperature. Observations of the separated components were taken in sunlight and UV-light, and following iodine fuming and exposure to Dragondorff's reagent.

Results & Discussion

TLC analysis was carried out to study the difference in the constituent profiles of these poisonous plant seeds. A marginal difference in constituent profiles of these samples was observed, which is because plants have their own distinctive chemical component profiles. TLC analysis method for the analysis of *Datura fastusa* revealed only three spots, which could be separated by using solvent system comprising methanol: water (70:30), and visualization was done by spraying Dragondorff's reagent.[2] Some solvent systems used for the TLC analysis of *Ricinus communis* have revealed only one spot at hRf 25 after separating this sample with a solvent system containing petroleum ether: diethyl ether: acetic acid (60:40:02). Spot with hRf value of about 25 has been obtained with the presence of castor oil (fixed oil obtained by cold expression from the seeds of *Ricinus communis*), and all the other spots were seen above this spot.[3] In the present work, forty solvent systems were tried (**Table 1**), out of which eight solvent systems [Benzene: Hexane: Methanol (60:30:10), Benzene: Hexane: Petroleum ether (60:30:10), Benzene: Hexane: Toluene (60:30:10), Benzene: Hexane: Acetone: Methanol (45:45:5:5), Hexane: Acetone: (80:20), Benzene: Toluene: Petroleum ether (80:10:10), Benzene: Hexane: Methanol (45:45:10)] gave useful results, and Benzene: Hexane: Ethanol (60:30:10) was the only solvent system that provided satisfactory separation (**Tables 2 & 3**).

Table 1: List of Solvent Systems Used in the Study

Solvent system	Proportions
1. Benzene: Hexane: Ethanol	(60:30:10)
2. Benzene: Hexane: Methanol	(60:30:10)
3. Benzene: Hexane: Petroleum Ether	(60:30:10)
4. Benzene: Toluene	(80:20)
5. Amyl alcohol: Acetone: Water: Ammonia	(50:50:30:0.4)
6. Benzene: Ethanol	(60:40)
7. Methanol: Water	(70:30)
8. Chloroform: Methanol	(98:2)
9. Amyl alcohol: Acetone: Water: Ammonia	(60:60:10:4)
10. Hexane: Acetone: Toluene	(40:40:20)
11. Chloroform: Methanol: Water	(80:15:5)
12. Cyclohexane: Chloroform	(70:30)
13. n-Hexane	Absolute
14. Petroleum ether	Absolute
15. Hexane: Acetone	(70:30)
16. Chloroform	Absolute
17. Acetone: 1-2, dichloroethane	(70:30)
18. Chloroform: Ethanol	(95:5)
19. Hexane: Acetone: Toluene	(45:45:10)
20. Acetone: 1-2dichloroethane	(60:40)
21. 1-2dichloroethane:Acetone	(70:30)
22. Benzene: Acetone: Methanol	(70:20:10)
23. Benzene: Acetone: Methanol	(80:10:10)
24. Benzene: Hexane: Methanol	(45:45:10)
25. Benzene: Toluene: Petroleum ether	(80:20:10)
26. Benzene: Hexane: Acetone: Methanol	(45:45:5:5)
27. Benzene: Hexane: Acetone: Methanol	(60:30:5:5)

Solvent system	Proportions
28. Xylene: Benzene: Petroleum ether	(40:40:20)
29. Cyclohexane: Toluene: Diethyl amine	(75:15:10)
30. Benzene: Acetone: Ethanol	(70:20:10)
31. Cyclohexane: Toluene: Diethyl amine	(75:10:15)
32. Water: Methanol: Acetone: Chloroform	(20:20:10:40)
33. Glacial acetic acid: Ethanol: Water	(30:60:10)
34. Chloroform: Water: Methanol: Acetone	(50:20:20:10)
35. Ethyl acetate: Methanol: Ammonia	(85:10:5)
36. Benzene: Ethanol	(80:20)
37. n-Hexane: Methanol	(80:20)
38. Toluene: Acetone: Methanol	(70:20:10)
39. Hexane: Acetone	(80:20)
40. Benzene: Toluene: Petroleum ether	(80:10:10)

Table 2: hRf Values of the Study - Experimental Conditions

Experimental Conditions	
Solvent System	*Benzene: Hexane: Ethanol (63:30:10)*
Saturation Time	*20 minutes*
Run Time	*29 minutes*
Temperature	*38°C*

Table 3: hRf Values of the Study

Sample Name	*Ricinus communis*	*Croton tiglium*	*Cerbera thevetia*	*Datura fastuosa*
No. of spots	**10**	**11**	**08**	**10**
HRf value	*30* *39* *43* *55* *57* *70* *76* *79* *82* *86*	*13* *15* *27* *29* *33* *39* *24* *45* *50* *86* *95*	*15* *18* *20* *29* *76* *92* *96* *98*	*10* *12* *15* *22* *25* *29* *32* *76* *96* *98*

All spots were made visible under iodine fuming

Conclusions

In this study, methanol and petroleum ether have been found to be the best solvents for the proper extraction of the selected samples. Forty solvent systems were tried, and only Benzene: Hexane: Ethanol (60:30:10) could produce fruitful and reproducible results. The constituents of the seeds of all the poisonous plant species undertaken in the present study can be separated and differentiated for the purpose of identification by this method. Spots were visualized best under iodine fuming, and satisfactory results were found lacking with daylight, UV light, and Dragondorff's spray.

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