Pharmacognostic and Phytochemical Screening of Datura stramonium by TLC and GC-MS: A Forensic Approach

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Forensic Botany helps in linking the location, type of crime, revealing modus Operandi, culprit as well as victim by various circumstantial facts. In the study, Datura stramonium was considered as major plant of Bundelkhand region encountered in poisoning cases in criminal Investigations. Datura plant was identified and its fresh leaves were washed to remove dirt, stored at 40°C and were subjected to microscopic examination for botanical features, preliminary screening for alkaloids identification and chromatographic techniques such as TLC and GCMS for qualitative analysis of toxins present in the plant. The result showed that all techniques applied are suitable to identify the plant through botanical derived evidences such as leaf traces, resins, or plant extracts during the crime scene investigations. GCMS technique identified the main toxins and resulted that it contains high concentration of hyoscine and very fatal when administered eventually causes death.

Keywords: Datura Stramonium; Forensic Toxicology; GC-MS; Phytochemicals; TLC.
Taxonomical Features of Datura

Datura stramonium belongs to family solanaceae, a nightshade family. Its origin is basically in North America. Its name was given by Carl Linnaeus also known as father of taxonomy. Leaves of it are coarse wavy at margin and are generally broad in length. Roots are long branched and thick. The fruit of Datura stramonium is oval in shape and thorny in nature which contains many dark brown seeds. Flower of the plant is funnel shaped and generally white in colour.

Toxicity of Datura Plant

The Datura plant is very toxic in nature therefore the chances of poisoning is more abundant during criminal investigations. The Datura plant has toxic components in its all parts like leaves, seeds, stem and flowers. The main toxic components of the Datura are scopolamine/Hyoscine, Atropine. 50/100 seeds contain 3-6mg of atropine and each seed contains 0.1mg of atropine.

Pharmacognostic effects of Hyoscine/Scopolamine

When hyoscine/scopalamine as main constituent is administered in body has some adverse effects such as Nausea, Headache, unconsciousness, ulceration, high blood pressure, unable to breath, weakness, fatigue, are some common early symptoms. Long effects such as Tachycardia, Dementia, Arrhythmia, Urinary retention, Blurred vision and dry mouth drowsiness.

MATERIALS AND METHODS

Collection of Plant

The plant Datura stramonium fresh leaves were collected from Jhansi city of Bundelkhand region 25.4484° N Longitude and 78.5685° E

Fig. 1. Fresh Leaves and Stem of Datura Stramonium

Fig. 2. Dried Fruit of Thorn Plant Containing Seeds Structure of Hyoscyamine

Fig. 3. Crushing by Pestle and Motar and Extract of leaves in glass bottle
Latitude at normal 30°C and Wind N at 3 km/h, and 80% Humidity with help of weather vane and hygrometer in the month of May. The plant was identified and described taxonomy in Department of Botany, Bundelkhand University, Jhansi. In the present study, the fresh leaves were washed to

**Fig. 4.** The model (PerkinElmer Clarus 680) GC-MS was used for chromatographic analysis

**Fig. 5.** A. Abaxial and adaxial View of Leaf and B. Microscopic View of Leaf

**Qualitative Report**

File: C:\TurboMass\BUNDLKHAND UNIVERSITY.PRO\Data\D2.raw
Acquired: 16-Oct-20 12:55:26 PM
Description: GC: 22022016_Arjuna.mth MS: Rambir 31018.EXP
Sample ID: BYAD2
Printed: 16-Oct-20 11:57 PM
Page 1 of 1
Vial Number: 92

**Graph 1.** Shows the data of peaks obtained through GC of Datura stramonium
remove dirt and stored at 4°C in aluminium foil. The following examinations were done.

Microscopic and Macroscopic examination

Fresh leaves and stem of the plant was considered for the microscopic examination for species identification of the plant found in Bundelkhand region. Physical parameters of leaf, stem of plant were noted for species Identification.

| Serial No | Operation     | Running Information               |
|-----------|---------------|-----------------------------------|
| 1         | Oven          | Initial temp 40°C for 5 min       |
| 2         | Ramp          | 12°C/min to 260°C                 |
| 3         | Hold          | 10 min                            |
| 4         | Injection B Auto | 250°C                      |
| 5         | Volume        | 0.1 L                             |
| 6         | Split         | 50:1                              |
| 7         | Carrier Gas   | He                                |
| 8         | Solvent Delay | 2.00 min                          |
| 9         | Transfer Temp | 180°C                             |
| 10        | Source Temp   | 200°C                             |
| 11        | Column        | 30.0m x 250m                      |

Phytochemical study of plant

Fresh leaves of Datura plant were crushed through pestle and motor method and filtered by muslin cloth and then by Whatman filter paper. Prepared extract was used for phytochemical study to identify the presence of phytochemicals. Phytochemical study of plant was analysed for presence of alkaloids, sponins, anthraquinones, flavonoids, glycosides. Presence of glycosides and other highly poly complex structures may show the toxicity of the plant through phytochemical study.

Preparation of extracts-The extract of plant collected after filtration from muslin cloth, extract was dissolved uniformly in two different solvents such as N-Butanol and N-Hexane for Phytochemical screening. Different chemical test were performed

Chromatographic methods

TLC technique was applied for preliminary identification of toxic compounds such as Scopolamine/ Hyoscyamine. Atropine as major constituents for toxicity of plant. In this study hyoscine was detected through thin layer chromatography.

| Serial No | Chemical Test | N-Butanol Extract | N-Hexane Extract |
|-----------|---------------|-------------------|------------------|
| 1         | Alkaloids     | +                 | +                |
| 2         | Amino Acid    | +                 | +                |
| 3         | Anthraquinones| -                 | -                |
| 4         | Glycosides    | +                 | +                |
| 5         | Sponins       | -                 | -                |
| 6         | Flavonoids    | +                 | +                |

Note- Sign + {Present}, Sign- {Absent}

Fig. 6. Structure of hyoscyamine (C17H23NO3)/ Daturine identified in the extract of Datura leaves by GC-MS at RT 23.016 with 2.509 peak %. (https://pubchem.ncbi.nlm.nih.gov/image/imgsrv.fcgi?cid=154417&l=1)
chromatography TLC. Hyoscyamine-N- butyl bromide standard Rf value was compared with the extracts of the plant leaves. In different solvent systems, chloroform: ethanol (3:1) was used for visualization of alkaloids on the plate and Rf values were calculated.

### Table: Compounds Identified

| Serial No. | RT Peak | Peak % | Compounds Identified |
|------------|---------|--------|----------------------|
| 1          | 4.995   | 1.505  | Hematoporphyrin, Tungsten, Aconitane-1 |
| 2          | 6.821   | 1.307  | Hematoporphyrin      |
| 3          | 9.912   | 2.365  | O-methylxime Delsoline, Methanesulfonic acid,  |
| 4          | 12.915  | 2.770  | 3-Pyridinecarboxylic acid, Hematoporphyrin Lycophyll, Rhodopin |
| 5          | 16.118  | 3.572  | Decanoic acid, Docosanoic acid,  |
| 6          | 18.867  | 1.603  | Trilinolein, L-Proline, 5,5\(^{-}\)bis(trimethylstannyl)  |
| 7          | 21.703  | 1.004  | Octadecane, 3-ethyl-5-(2-ethylbutyl), Lanosta-7,9(11)-dien-18-oic acid, Tetracosane, 12-decyl-12-nonyl-  |
| 8          | 21.770  | 1.168  | Cholestanol[7,8-\(\alpha\)]cyclobutane, 3-methoxy-6-oxo-2'-methylene-, Carotene, 3',4'-didehydro-1',2'-dihydro-1',2'-dihydroxy-, (2'R), Cinobufotalin  |
| 9          | 21.888  | 1.061  | D-Glucopyranoside, Tungsten, 1,2-bis(dimethylphosphino)ethane  |
| 10         | 22.051  | 1.086  | cis-Vaccenic acid, Octadecenoic acid, cis-13-Eicosenoic acid, cis-10-Nonadecenoic acid  |
| 11         | 23.016  | 2.509  | Atropine, O-Bromoatropine, Hyoscyamine, Atropine, acetate  |
| 12         | 23.502  | 1.233  | Oleic acid, eicosyl ester, 17-Pentatriacontene, 9-Octadecene, 1-[2-(octadeccyl)oxy]ethoxy]  |
| 13         | 24.008  | 2.232  | Scopolamine, Benzeneacetic acid, Cyclopropanebutyric acid,  |
| 14         | 24.780  | 1.157  | 2-Nonadecanone 2,4-dinitrophenylhydrazine, Pentatriacontene, Tetrapentacontane, 1,54-dibromo-, 3-Desoxo-3, 16-dihydroxy-12-desoxyphorbol 3,13,16,20-tetraacetate  |
| 15         | 25.891  | 2.355  | Rhodoxanthin, 4'-Apo-\(\alpha\),\(\psi\)-carotenoid acid  |
| 16         | 28.918  | 1.108  | 2,4,6-Decatrienoic acid, Aconitane, trimethylsilyl ester,  |
| 17         | 29.053  | 1.562  | Cholestanol, 3,5-dichloro-6-nitro-, 6-methyl-hept-5-enolic acid, methyl ester  |
| 18         | 29.348  | 1.880  | D-Glucopyranosiduronic acid, Cholan-24-oic acidMethyl cholate  |
| 19         | 29.457  | 1.397  | 1,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis(1-trimethylsilyl)propyl disiloxane, 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol  |
| 20         | 29.635  | 1.064  | dicarbonyl-(\(\psi\)-4-2-methylenecycloheptanone)(1,2-bis(dimethylphosphino) ethane), 1',1'-Dicarboethoxy-1\(\alpha\),  |

**Graph 2.** Shows MS spectrum of (RT Peak 23.016) m/z for identification of Hyoscyamine compound with data reference from NIST Library and main library of GCMS.
RESULTS

The TLC chromatogram obtained after the calculation of Rf value and was matched with standard value {0.81}. The extract shows the presence of hyoscyamine. Hyoscyamine is most lethal component found in Datura stramonium.

CONCLUSION

The Phytochemical screening of plant in different extracts showed the presence of alkaloids, saponins, flavonoids and amino acids. The alkaloid spots were identified by TLC. The spectral data of GC-MS confirmed the presence of different compounds such Atropine, scopolamine, hyoscyamine and their chemical characterization. This study substantiate with the previous literature that the leaves of Datura stramonium are toxic in nature. In cases of poisoning by Datura stramonium in Bundelkhand region, the plant can be easily identified through botanical derived evidences such as leaf traces or plant extracts during the investigations of crime scene and is very fatal (1471 ug/kg) in men and eventually cause death, and contains high concentration of major tropane alkaloids as hyoscyamine and scopolamine.

Conflict of Interest

There is no conflict of interest including honorarium, grants, membership, employment, ownership of stock or any other interest or non financial interest such as personal or professional relation, affiliation and knowledge of the research topic between the authors.

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