

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 40C 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.

Forensic Botany deals with the study of the plant science and is derived as botanical evidences in court of law for criminal administration purposes. From the historical point of view, plants and its extracts has been used as important tool as modus operandi in crimes such as, human poisoning, cattle poisoning and cattle revenge etc. Such type of botanical evidences can be found in suicidal, homicidal and accidental poisoning¹. The plant derived botanical evidences may be Pollens grains, leaves, stem root, sap, resins and others encountered during crime scene investigations.

Forensic Botany helps in linking the location, type of crime, revealing modus Operandi, culprit as well as victim by various circumstantial

facts. There are various toxic plants grow very easily as aggressive weed in the temperate regions such as *Datura stramonium*, *Abrus preatorious*, *Argemone mexicana*, *Jatropha curcas*, *Ricinus communis*, *Nerium oleander* etc. In the present study, *Datura stramonium* has been considered as major plant encountered in poisoning cases in criminal investigations².

Datura stramonium also called as Jimson weed, angels trumpet, thorn apple, and devil's trumpet and many other names is an annual poisonous plant. Its seeds, leaves, roots almost whole plant is poisonous and contains various poisonous compounds like atropine, hyoscyamine, and scopolamine³.

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 thick⁴. 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 investigations⁵. The *Datura* plant has toxic components in its all parts like leaves, seeds, stem and flowers⁶. The main toxic components of the *Datura* are scopolamine/Hyoscine, Atropine⁷. 50/100 seeds contain 3-6mg of atropine and each seed contains 0.1mg of atropine⁸.

Pharmacognostic effects of Hyoscine/Scopolamine

When hyoscine/scopolamine 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 symptoms⁹. Long effects such as Tachycardia, Dementia, Arrhythmia, Urinary retention, Blurred vision and dry mouth drowsiness¹⁰.

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

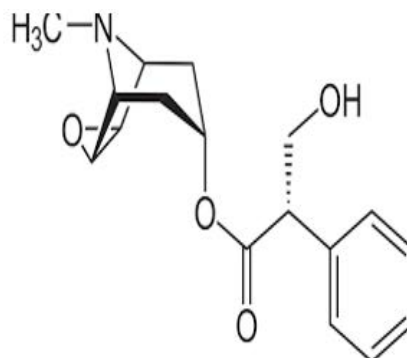


Fig. 3. Crushing by Pestle and Motar and Extract of leaves in glass bottle

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



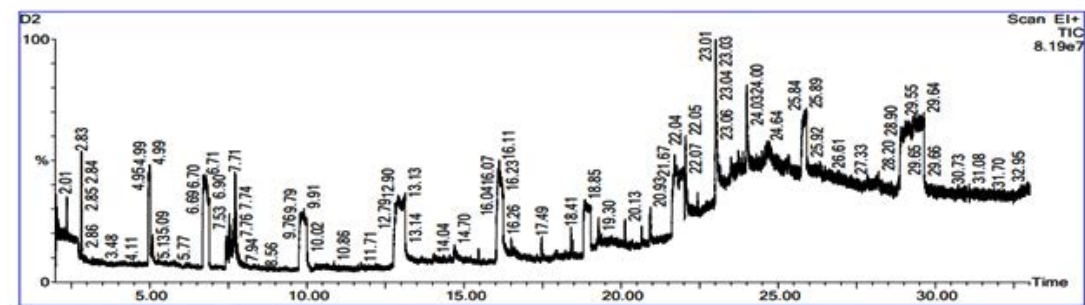
Fig. 4. The model (PerkinElmer Clarus 680) GC-MS was used for chromatographic analysis



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

Qualitative Report

File: C:\TurboMass\BUNDELKHAND UNIVERSITY.PRO\Data\D2.raw
 Acquired: 16-Oct-20 12:55:26 PM
 Description:
 GC/MS Method: 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.

Table 1. The Acquisition Parameters Used in Operating the GC-MS

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 iL
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 250im

Table 2. The Microscopic Properties of Plant

Properties	Observation
Color	Ash Green
Shape	Broadly palmate
Size	10-15 cm. x 7.5-12.5 cm
Apex	Pointed
Venation	Reticulate
Margin	Cordate

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 phytochemicals¹¹. Phytochemical study of plant were 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 study¹².

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/ Hyoscyamine¹³. Atropine as major constituents for toxicity of plant. In this study hyoscyine was detected through thin layer

Table 3. Phytochemical Screening of Plant in Different Extracts

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

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

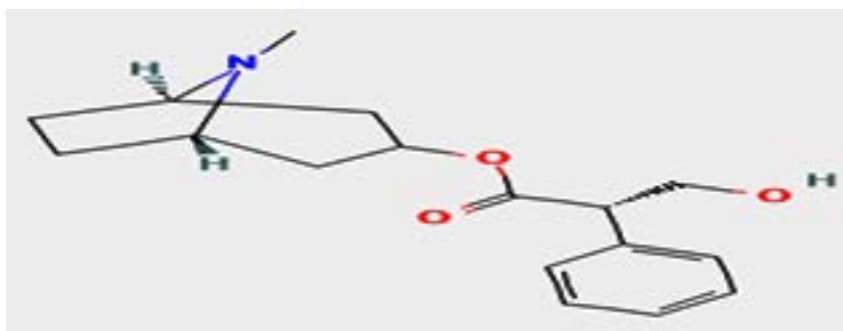


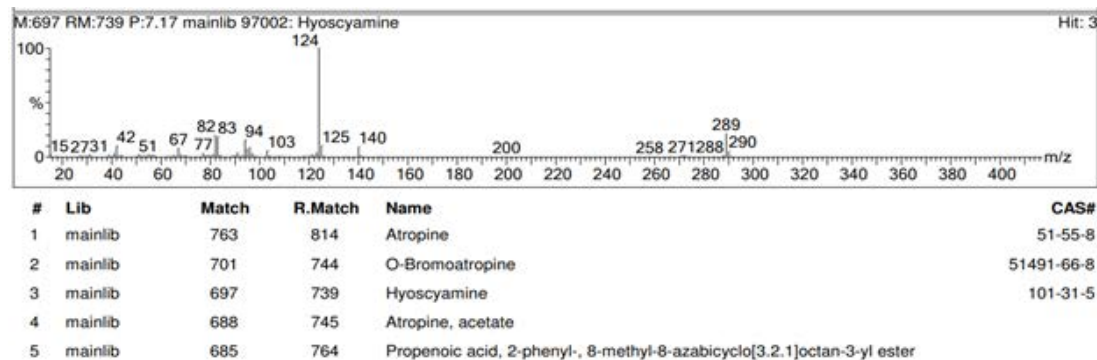
Fig. 6. Structure of hyoscyamine (C₁₇H₂₃NO₃)/ Daturine identified in the extract of Datura leaves by GC-MS at RT 23.016 with 2.509 peak %. (<https://pubchem.ncbi.nlm.nih.gov/image/imgsrv.fcgi?cid=154417&t=1>)

chromatography TLC. Hyoscyamine-N- butyl bromide standard Rf value was compared with the extracts of the plant leaves¹⁴. In different solvent systems, chloroform: ethanol: water (81:11:8),

chloroform: ethanol (3:1)¹⁵. The TLC plate was runned after spotting. Dragendroff reagent was used for visualization of alkaloids on the plate and Rf values were caluculated¹⁶.

Interpretation of GC-MS Chromatogram

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-methyloxime 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	Cholestano[7,8-a]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-(octadecyloxy)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- α , ψ -carotenoic acid
16.	28.918	1.108	2,4,6-Decatrienoic acid, Aconitane, trimethylsilylester,
17.	29.053	1.562	Cholestane, 3,5-dichloro-6-nitro-, 6-methyl-hept-5-enoic acid, methyl ester
18.	29.348	1.880	D-Glucopyranosiduronic acid, Cholan-24-oic acid Methyl cholate
19.	29.457	1.397	1,3-Dichloro-1,3-bis(norbornadien-2-yl)-1,3-bis(3-trimethylsilylpropyl) disiloxane, 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol
20.	29.635	1.064	dicarbonyl-(α -4-2-methylenecycloheptanone)[1,2-bis(dimethylphosphino) ethane], 1',1'-Dicarboethoxy-1 α ,



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 as 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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