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Phyto-Chemical Analysis of Tropane Alkaloids In Datura Poisoning

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ABSTRACT:

Traditionally Datura plants have been used for mystic and religious purposes and as natural drugs with narcotic effects or to treat asthma .Several accidental intoxications of humans and animals coming from food sources contaminated with Datura plants have also been reported. The seeds of this plant are some time given to children with a view to kidnap them when they become unconscious or delirious. A decoction of the seeds is at times added to liquor or toddy with a view to enhance its intoxicating property. seeds are employed mainly as a stupefying poison prior to robbery, kidnapping and rape Crushed seeds are also mixed in Prasad (Laddoo) in India. Cases related to datura poisoning are generally referred to forensic laboratories for their identification along with viscera and gastric lavage, for toxicological analysis. Survey of literature reveals that different techniques for its identification have been recommended and scattered in the scientific literature. In the present paper efforts have been made to review the selected techniques of the identification of its alkaloids. Phytochemical, Chromatographic and Spectroscopic methods of analysis suggested by various workers from time to time have been reviewed. Methods of analyzing powdered datura seeds have also been included in this paper with the view to compile the scattered literature and to provide an up to date comprehensive ready reference document to the forensic toxicologist & chemists.

Key Words- Datura poisoning, Tropane alkaloids, Phytochemical analysis, Review of analytical techniques

INTRODUCTION

The name *Datura* comes from the early Sanskrit Dustura1 or dahatura. *Datura* is referred to an ancient Indian literature as Shivashehara because the flowers are belived to be associated with Lord Shiva. Common names for datura are numerous, some of the most common ones being raving nightshade, thorn apple, stinkweed, Devil's apple, Jimson weed, and angel's trumpet (1,2),In India it has been referred and attributed to as "Poisonous" and aphrodisiac. In little measures it was used in Ayurveda as a medicine from the ancient times. It is used in rituals and prayers to Shiva. Most parts of the plants contain toxic hallucinogens and datura has a long history of use for causing delirious states and death. It was well known as an essential ingredient of love potions and witches' brews.(3) All datura plants contain tropane alkaloids such as scopolamine, hyoscyamine and atropine, primarily in their seeds and flowers. Because of the presence of these substances, datura has been used for centuries in

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some cultures as a poison and hallucinogen The datura species have been described in detail by Kirtikar and Basu (4), Chopra et al (5) and Evans(6).

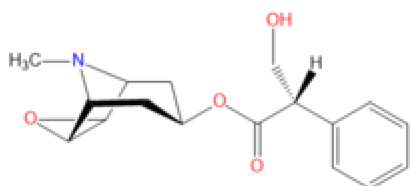
BOTANICAL FEATURES:

The genus datura belongs to the Solanaceae family, which is well known for synthesizing a number of tropane alkaloids. D*atura* are herbaceous, leafy annuals approximately 2 meters in height. The leaves are alternate,10- 20cm long and 5-18cm broad .The flowers are erect or spreading ,trumpet shaped ,5-20cm long and 4-12cm broad at the mouth, colours vary from white to yellow ,pink and pale purple, fruit is a spiny capsule 4-10cm long and 2-6cm broad, splitting open when ripe to release the numerous seeds The whole plant is antiseptic, narcotic, sedative and is useful for asthma(7) , leaves narcotic and antispasmodic. Traditionally datura plants have been used for mystic and religious purposes (8) and as natural drugs with narcotic effects or to treat asthma [9]. Well known psychoactive effects make datura a tempting choice for sensation-seeking young people. Plants are consumed or smoked to achieve hallucinogenic experiences [10-13]. On the other hand, several accidental intoxications of humans and animals coming from food sources contaminated with datura plants have also been reported [14,15 16]. In some parts of Europe and India, *datura* has been a popular poison for suicide and murder. From 1950 to 1965, the State Chemical Laboratories in Agra, India, investigated 2,778 deaths caused by ingesting *datura.* In some parts of Europe and India, *datura* has been a popular poison for suicide and murder. From 1950 to 1965, the State Chemical Laboratories in Agra, India, investigated 2,778 deaths caused by ingesting *Datura.*(17). Medicolegal aspects of datura species have been described by Subrahmanyam (18) , Parikh(19) and Goutam & Goutam.(20)

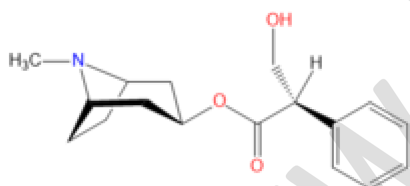
The seeds are some time given to children with a view to kidnap them when they become unconscious or delirious. The seeds are given whole or more often crushed ,mixed with rice, al(pulse),sweets, Chaptis or vegetables and some time with tea, coffee or liquor. The seeds as well as eaves are also mixed with tobacco or ganja and smoked in a chillum (pipe) for the same purpose. A decoction of the seeds is at times added to liquor or toddy with a view to enhance its intoxicating property.(21) To analyze the articles related to datura poisoning like datura fruits and seeds (whole or crushed), poisoned food materials(sweets, Pradad etc),drinks(Tea, coffee ,toddy, liquor) or any other kind of ayurvedic drugs containing datura as one of the component (in case of over dose or accidental poisoning), Chilam if datura has been given with tobacco or ganja for smoking ,these are referred to forensic laboratory for their identification along with viscera and gastric lavage, for toxicological analysis. Therefore efforts have been made to review the various techniques which can be useful for the identification of datura and its active components tropane alkaloids. Phyto-chemical analysis,, Chromatographic separation & characterization by TLC, HPTLC, HPLC , LC-MS and Spectroscopic analysis suggested from time to time have been reviewed. Macroscopic & Microscopic methods of analyzing Dhatura alkaloids and powdered datura seeds have also been included in this paper. Related scattered literature compiled, to provide an up to date ready reference to the Toxicologist and Forensic chemists with the aim it may be an useful tool to the scientist for the selection of the methodology.

Tropane alkaloids of Datura spp:

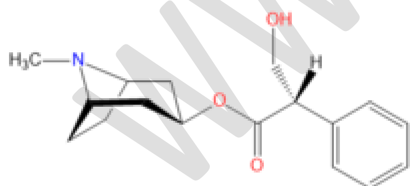
The term tropane alkaloids refers to a group of more than 200 compounds best known for their occurrence in the family *Solanaceae* comprising over 100 genera and 3000 plant species. They have in common a two-ringed structure characterized by a pyrrolidine and a piperidine ring sharing a single nitrogen atom and two carbons atoms. The nitrogen atom at the end of the molecule, which characterizes the compounds as alkaloids, is in this group characteristically methylated. The most important natural tropane alkaloids are (-)-hyoscyamine and (-)-scopolamine (also known as hyoscine). High concentrations of these alkaloids have been found particularly in *Datura stramonium* and *Datura ferox,* as well as in *Datura innoxia.* The pattern of tropane alkaloids differs significantly and in *Datura stramonium* (also known as thorn apple or Jimson weed) hyoscyamine prevails in most parts of the plant, whereas in *Datura ferox* scopolamine is the major alkaloidproduced. *Datura* plants are toxic for animals if ingested in larger amounts. Their seeds, which contain significant amounts of hyoscyamine and scopolamine, can be found as botanical impurities in feed materials, particularly in soybean and linseed products.(22.)



Scopolamine



Atropine



Hyoscyamine

Structural formula of the main constituents of Datura

Review of the analytical techniques:

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Phytochemical analysis-

Colour tests –

Most alkaloids are precipitated from neutral or slightly acid solution by Mayer's reagent (potassiomercuric iodide solution), by Wagner's reagent (solution of iodine in potassium iodide), by solution of tannic acid, by Hager's reagent (a saturated solution of picric acid), or by Dragendorff's reagent (solution of potassium bismuth iodide). These precipitates may be amorphous or crystalline and are of various colours: cream (Mayer's), yellow (Hager's), reddish-brown (Wagner's and Dragendorff's).(23)

Some other specific colour tests are as follows(24)

(A) ***Vitali-Morin Colour Reactio)***: A few mg of hyoscyamine (and also atropine) is treated with about 0.2 ml of fuming HNO_3, evaporated to dryness on the water-bath. To the residue is then added 0.5 ml of a 3% (w/v) solution of KOH in methanol, it gives a bright purple colouration, that changes to red and finally fades to colourless.

(*a*) The 3% solution of KOH must be freshly prepared.

(*b*) The reaction is very sensitive i.e., up to 0.0001 mg of any of the alkaloids viz., strychnine, apomorphine, veratrine, physostigmine etc. give a positive test.

(B) **para-Dimethylaminobenzaldehyde Reagent**: [Prepared by dissolving 2 g of*p*-Dimethylaminobenzaldehyde in 6 g of H_2SO_4 to which 0.4 ml of water is added previously]. Add to 5-10 mg of hyoscyamine in an evaporating dish 2-3 drops of this reagent and heat on a boiling water-bath for several minutes. A distinct red colouration is produced that ultimately gets changed to permanent cherry red upon cooling.

(C) ***Gerrard' s test*** **:-** If one or two cubic centimeters of 2% sol of mercuric chloride in 50% alcohol are added to a portion of the of the residue, a red colour develops immediately Hyosscyamine produces (Hyocine) does not produce any change in colour.

Crystal tests

(I) Precipitation test:- An aqueous solution of hydrobromic acid saturated with bromine produces a yellow amorphous precipitate which after a short time forms crystals of various form such as spindles, crosses and stars.

(II) Gold chloride :- gives a citron yellow precipitate to a solution containing atropine if the precipitate be recrystallized from boiling distilled water and acidified with hydrochloric acid it will show a minutely crystalline appearance and when dry will appear dull and pulverulent. It has a melting point of $137^{o} – 139^{0}$ C

Thin layer chromatographic analysis[25]

Different solvent systems have been tried/ recommended for the identification of tropane alkaloids. Some of the selected methods are as follows

Table I Rf. Values

	Solvent System			
Compound	I[26]	II[26]	III[26]	IV[27]
Atropine/ Hyoscyamine	0.39	0.45	0.21	0.38
Homatropine	0.43	0.53	0.28	0.37
Scopolamine	0.52	0.65	0.33	0.56

Plates – Silica gel G, 250 µm thick

Solvent Systems :-

System I- Chloroform : tetrahydrofuran : diethylamine (80:10:10).
System II – Chloroform, diethylamine (90:10).
System III– Chloroform : Cyclohexane : diethylamine (70:20:10).
System IV- Chloroform : Acetone : diethylamine (50:40:10)
Visualization: Spray with acidified iodoplatinate reagent.

Table II Rf. Values

Solvent system-	**I** [28]	**II** [28]	**III** [28]	**IV** [28]	V [28]	**VI** [29]	**VII** [29]	**VII** [29]	**IX** [29]
Layer-	**S**	**S**	**S**	**A**	**S**	**S**	**S**	**S**	**C**
Atropine	38	40	16	10	17	17	37	36	15
Hamatropine	37	45	15	24	15	---	---	----	----
Apoatropine	54	67	40	40	16	---	44	44	74
Belladonine	---	---	---	---	----	----	26	17	69
Scopolamine	56	60	19	00	52	---	73	83	53
Scopoline	60	90	44	50	37	---	---	---	---

Plates layer[30] : S = Silica gel G : A = alumina : C = cellulose powder
Solvent systems
I Chloroform : Acetone : diethylamine (50:4010). **II.** Chloroform : diethylamine (90:10)
III. Cyclohexane : Chloroform : diethylamine (50:4010), **IV**. Cyclohexane : Chloroform (30:70)+ 0.05 diethylamine.,**V**. Methanol, **VI**. Methanol : Acetone : triethanolamine (50:50:1:5)
VII. Diethylformamide : diethylamine :ethanol : ethyl acetate (5:5:30:60)
VIII. 70% Ethanol: 25 : ammonium hydroxide (95:1)
IX. 1. run : 15 cm., Heptane : diethylamine (100:0.2), 2run : 10 cm, benzene : heptane : Chloroform : diethylamine (60:50:10:0.2)

Location reagents – Dragendorff spray / Acidified iodoplatinate solution

Gas Chromatographic method

Analysis of tropane alkaloids e.g. Atropine, Homatropine and Scopolamine carried out using Gas Chromatographic methods. Ardrey and Moffat(31) examined the Atropine, Homatropine

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and Scopolamine using packed column2.5% SE30 on 80 to 100 mesh Chromosorb G and another method have been suggested in the Clark's Analysis of Drugs and Poison(32).The experimental conditions followed were as follows.(System I ,II& III).

System[33] I	
Packed column	2.5% SE30 on 80 to 100 mesh Chromosorb G
Column temperature	Normally between 100° c and 300° c ; for isothermal conditions, an approximate guide to temperature to use is the RI ÷ 10.
Carrier gas	Nitrogen at 45ml/min.
Retention indices	Atropine- 2199 Homatropine- 2072 Scopolamine (hyoscyamine)- 2192
System[34]II	
Capillary column	20 to 30 m x 0.2 or 0.25 mm i.d., 5%- phenyl- 95% - dimethyl – PSX (X-5) with a 0.5 to 1 µm film thickness.
Carrier gas	Helium, constant flow 1 ml/min.
Temperature programme	0.7 min at 90° c, 35° 1 min to 240° c, 8° /min to 290° , 25° c min to 325° c, 6 min final hold.
Reference compounds	n- alkanes with an even number of carbon atoms.
Retention indices	
Atropine	2293
Homatropine	2165
Scololamine (hyoscyamine)	2427

A method of identifying these alkaloids the Analytical Manual , Drug Enforcement Administration,USA ,recommended a method using Flame Ionisation detector and Glass coloum with 3% OV-1on chrom WHP 80/100 .Experimental conditions are as follows

System[35] III	
Detector	Flame ionization
Column	Glass(2ft X 4mm ID)
Packing	3% OV-1 on chrom WHP 80/100 mesh
Carrier gas	Nitrogen
Flow rate	25 ml/min
Injector temp.	250° c
Column temp.	250° c
Detector temp.	250° c
Compound	Retention time (minutes)
Homatropine	1.3
Atropine/Hyoscyamine	2.0
Scopolamine	3.2

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HPLC Analysis

Following are three HPLC methods of analysis of tropane alkaloids suggested [36,37,38] using the different experimental conditions. Details are given in the following tables.

HPLC Analysis	
Method[36] I	
Apparatus	HPLC low- pressure ternary gradient system.
Column	Reversed- phase, Lichrosorb C_8, 250 X 4mm ID
Mobile phase	Acetonitrile/ aqueous sodium dodecylsulfate. (30:70 V/V)
Flow rate	2.5 ml min^{-1}, 2500 psi
Injection size	20 µl containing 2 µg
Detection UV	ƛ = 220 nm, 0.08 AUFS
Retention times	Min
Atropine	5.50
scopolamine	17.42

Method[37] II	
Column	Silica (Spherisorb S5W, 51 J.m, 12.5 cm x 4.9 mm internal diameter).
Eluent	A solution containing 1.175 g (0.01M) 0f ammonium perchlorate in 1000 ml of methanol; adjust to pH 6.7 by the addition of 1ml of 0.1M sodium hydroxide in methanol.
K' values	Atropine- 3.9 (tailing peak). Hyoscine. 1.1 Homatropine – 4.2 (tailing peak).
Method[38] III	
Column	C_8 Symmetry (250 x 4.6i.d, 5µm). with Symmetry C_8 per column (20mm)
Column temp.	30° C
Mobile phase	(A:B) Phosphate buffer (Ph3.8):Acetonitrile.
Elution programme	(85: 15) for 6.5 min to (65:35) until 25 minto (20:80) for 3 min and back to initial conditions for equilibration for 7 min.
Flow rate	1 ml/ min for 6.5 min, thenlinear increseto 1.5 ml/ min for 6.5 to 25 min and hold for 3 min (re-equilibration is made at 1.5 ml/min)
Detection	UV diode –array
K' values	Atropine- 10.4, Hyoscine.- 7.4

Ashtiania and Sefidkonb(39) *d*etermined the datura alkaloids by high-performance liquid chromatography (HPLC) method. Samples were extracted with chloroform- methanol- cc. ammonia 15:15:1(v/v/v). HPLC separation was performed on two C8 columns. An isocratic mobile phase of acetonitrile- 50 mM phosphate buffer 10:90 and 20:80(v/v) was used. Peaks were identified by standards and diode-array detection. Scopolamine and atropine were determined by external method at 210 nm. Using Knauer and Teknokroma system equipped with a K-1001 pump and a manual injector. The UV detector was a 210 λmax and the column used was a packed with 25 × 0.46 cm Eurospher-100C8 (knauer, Germany, A) and Lichrospher 100 RP8 (Teknokroma, Spain, B), packed 5 µm particles. The isocratic mobile

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phase was a mixture of 10 and 20% acetonitrile and a buffer containing 50 mM sodium dihydrogen orthophosphoric acid, adjusted to pH 2.95 with orthophosphoric acid for A and B columns. Sample injection was 20 µl, and the analysis was performed at a flow rate of 0.8 and 1.0 ml/min for the 10 min, detection was conducted at 210 nm. The data were generated using a ChromeGate, employing atropine and (-) scopolamine as standard samples. Quantification of the alkaloids Quantitative determination was performed by external standard method. The standard solutions containing atropine and scopolamine (4, 10, 25, 50, 100, 200, 400 ppm) were prepared in methanol. A 20 µL volume of each standard solution was injected onto the HPLC column. They reported the calibration graphs for atropine and scopolamine constructed by plotting the peak area of the alkaloids versus their construction.

Analysis of tropane alkaloids,of two Atropa spp. scopolamine and atropine also examined and reported by Hosseini et al(40) using a reverse phase high-performance liquid chromatography (HPLC) equipped with UV-PDA detector. Extractions were carried out using a power sonic 405 (Hwashin Technologies, Korea) ultra sonic chamber. A pH-meter, model CG- 840 (Schott Gerate Gmbh, Germany) was employed to adjust pH in different stages. HPLC analyses were carried out on a C18 Lichrospher 100 column (5 µm, 250 x 4.6 mm) equipped with a K- 1001 pump, K-2800 UV-PDA detector, and a 20 µl injection loop; all from Knauer (Germany). A 10 mm C8 pre-column was coupled to the analytical column. The samples were analyzed using a buffer containing 50 mM potassium dihydrogen orthophosphoric acid adjusted to pH 3.0 by orthophosphoric acid: Acetonitrile (80:20 v/v). The mobile phase was pumped at a constant flow rate of 1.4 ml min-1 and detection was carried out at a wavelength of 215 nm. liquid chromatography–mass spectrometry(LC-MS).

Jakabová et al(41). analyzed datura alkaloids by LC-MS technique using a new generation of core-shell particle packed column. Tropane alkaloid content was investigated in various plant organs of four Datura taxa (D. innoxia, D. metel, D. stramonium, and D. stramonium var. tatula), grown under the same conditions, in two developmental stages. Authors developed a rapid LC-MS method for the quantitative determination of atropine and scopolamine, which was successfully applied to quantify the alkaloids in different plant organs (leaves, flowers, stems, seeds) of thorn apples after a simple sample preparation step. Elaboration and validation of the method and analysis of plant extracts were carried out by UFLC-MS technique, employing an Ascentis Express C18 column. Detection was done in positive ionization mode (ESI+) and the method suitability was evaluated by several validation characteristics. Quantitation limits reported 333 and 167 pgmL(-1) for scopolamine and atropine, respectively,

Borbala Boros et al(42) determined selected alkaloid components in the nectar of Datura species applying a simple and rapid liquid chromatography coupled with electrospray mass spectrometry analysis for the quantitative determination of atropine and scopolamine, used method allowed the direct coupling of an electrospray mass selective detector to the LC system. Applying these conditions, atropine and scopolamine were well separated from other components and detected with mass spec- trometry (mass selective detector). Simultaneous determination of atropine and scopolamine was also reported with gradient elution on an Ascentis Express C18(Supelco) reversed-phase column based on a new fused core particle design. Liquid chromatography coupled with electrospray mass spectrometry was used in

positive ion mode. Atropine and scopolamine produced protonated species at m/z 290 and 304.

Ricard F(43) et al reported the hair analysis in order to identify a possible consumption of a Datura seed infusion. After decontamination and washing, hair strands were segmented into four pieces and grinded into a fine and homogeneous powder. We they incubated 20 mg of fine and homogeneous hair powder for 10 min in 1 mL of phosphate buffer at pH 5.0 in the presence of 100 ng of ketamine-d4, used as internal standard (IS). Liquid-liquid extraction was performed with 4 mL of a mixture of hexane/ethyl acetate (1/1, v/v). The residue was reconstituted in 80 µL of mobile phase. A further 10 µL were injected into an 1.9 µm Hypersil GOLD PFP column (100 mm×2.1 mm) eluted with a gradient of acetonitrile and 2 mmol/L 0.1% formate buffer at a flow rate of 300 µL/min. Compounds were detected by a LCQ TSQ Vantage XP triple-quadripole mass spectrometer equipped with an electrospray ionization (ESI) source set in positive mode. SRM transitions m/z 290.2→124.1, m/z 304.2→138.1, and m/z 242.1→129.1 were optimized for atropine, scopolamine and IS, respectively.. Both atropine (from 8.4 to 15.0 pg/mg) and scopolamine (1.0-1.3 pg/mg) were identified in the four segment of the hair showing a regular consumption of Datura admitted by the patient himself

Macroscopic & Microscopic analysis Dhatura seeds.

Macroscopic & Microscopic analysis Dhatura seeds have been described in the Quality Standard of Indian medicinal Plants(44) however Macro & Microscopic studies of Datura metel Linn was reported by Chaudhuri et al(45) , Sarbadhikari and Gupta (46) and Wallis(47), It is also available in The Ayurvedic Pharmacopoeia of India.(48) According to Quality Standard of Indian medicinal Plants, macroscopically seeds of Datura metel are flattened ,ear shaped, slightly beaked towards the lower slide, about 4to5 mm long ,3to 4mmwide and 1to1.5 mm thick, pale brown, pitted with 2 to 3 prominent parallelly running concentric ridges along the thicker cocave margin of about 3/4th of the .circumference of the seed. Apale brown,2-2.5mm long fleshy strophiole lies towards themicropyle in the groove of the acute side. Odour faint disagreeable taste bitter and Microscopically Diagrammatic TS passing through the centre of seed is somewhat rectangular in shape with three ridged corners at the narrow endings, encircling a white endosperm embedded within which lies the spherical ending of cotyledons and radicle.

Datura seeds

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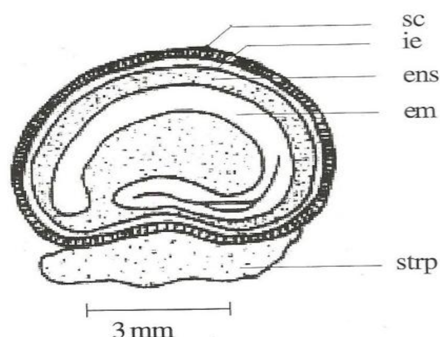
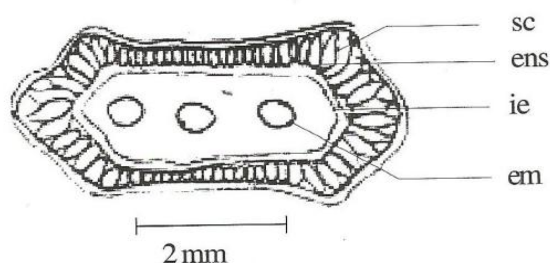
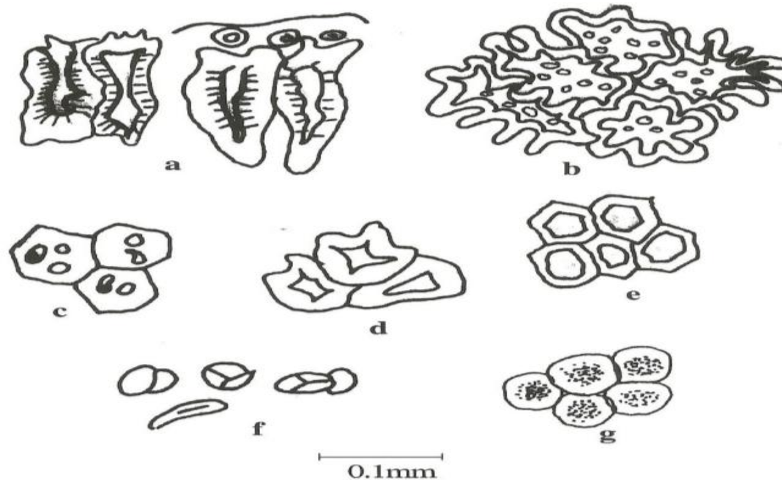




Diagrammatic LS of seed Diagrammatic TS of seed

Gupta et.al (44). reported the details of the TS of seed and mentioned that TS shows an outer sclerenchymatous layer of testa which is different in size and shape at the ridges corners from that of the straight faces lying in between them. Microscopic study of powered seed shows outer epidermis of testa in surface view with deeply sinuous folds, uniform thickening and pits; isolated or group of thick –walled transversely striated highly thickened palisade like cells of sclerenchyma and spherical to rectangular stone cells; plenty of starch grains of various sized and shapes from the strophiole; fragments of endosperm filled with fixed oil and aleurone grains; fragments of pigment layer in surface view; fragments of parenchymatous cells containing micro sphenoidal crystals.



(Source- Gupta et al(44). Quality Standard of Indian medicinal Plants)
Powder microscopy of datura metal seed. a. palisade like sclereids; b, fragment of testa in surface view; c, fragments of endosperm with oil globules and aleurone grains; d. stone cells; e, pigment layer in surface view ; f, starch grains from strophiole; g, parenchymatous cells with microsphenoidal crystals.

CONCLUSION

Review highlights different methods generally used for the identification of Datura alkaloids .On the basis of the availability of the instrumental facilities, selection of the method can be made and suspected evidence can be examined .If forensic report is based on chemical, chromatographic & botanical features that can not be challenged by any means. This review may be useful source material to the forensic toxicologist , forensic chemists as well as to forensic biologists.

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