

Pharmacognostic studies on seed of *Datura ferox* L.

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Received 27 August 2013; Accepted 27 August 2014

Datura ferox L. is an erect annual herb belonging to the family Solanaceae. Its seeds are useful in the treatment of delirium and mania. Present paper includes powder microscopic and organoleptic characters of the seeds. The physico-chemical parameters of raw drug, viz. extractive values, ash values, formulation, besides wt. per mL, total solids, alcohol content along with HPTLC finger printing and UV studies were undertaken on mother tincture for the first time.

Keywords: *Datura ferox* L. Pharmacognosy, Physico-chemical, Malphigian cells, HPTLC, Homoeopathy.

IPC code; Int. cl. (2014.01)–A61K 36/00

Introduction

Datura ferox L. commonly known as Chinese Datura is a stout bushy annual herb belonging to the family Solanaceae. It is a native of China introduced in Europe, Australia and in India. The leaves are alternate, ovate and coarsely sinuate dentate. Flowers erect, white, capsules straight, ovoid, spiny and long. Seeds small greyish black, bilaterally compressed, having undulated surface with testa reticulated and pitted. The outermost epidermis of testa possesses 1-layered lignified malphigian cells. A narrow zone of nucellus is present. The malphigian layer and nucellus become wider towards hilum. The seed endosperm has a single layered epidermis. The endosperm is precocious containing starch and oil. The embryo is conspicuous also with starch and oily substances. The seeds are useful in Homoeopathy for treatment of delirium and mania as mentioned in Waitz Prakf. Beob. Ub. Java. Arzum 1829 and Buchneri Toxicologie^{2,3}. Chemically the plant contains, scopolamine, hyoscyamine, atropine (Fig.1a, b, c), hyoscyne, meteloidue, datura lactone, daturalone, 3 α -tigloyloxytropane, 3-phenylacetoxyscopine, aposcopolamine, hygrine, 7 β -hydroxyl-6 β -propenyloxy-3 α -tropoloxytropane, 15 β -hydroxynicandrin B, nicandrin B, withanicandrin, withastramonolidine, withametalin E, 6- hydroxyl hyoscyamine, tigloytropine and 7-hydroxywithanolides^{4,7}.

Earlier studies on *Datura ferox* L. pertaining to pharmacognostic and physico-chemical parameters in general and in homoeopathic perspective in particular are not available. Hence, the authors have undertaken these studies for the first time as per the protocols suggested by Central Council for Research in Homoeopathy (CCRH), Government of India.

Materials and Methods

Pharmacognosy

The seeds of *D. ferox* L. were obtained from Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu (Access No. 8666, collected at Kendhala by D. Suresh Baburaj). The seeds were boiled at 80° C for 10 minutes, cooled and fixed in Formalin-Acetic acid-Alcohol F.A.A.). Later, they were dehydrated through Tris Buffered Saline (TBS) series and embedded in paraffin wax. Sections cut between 8-10 microns were stained with crystal violet – basic fuchsin combination⁸. The microscopic characters of powder were observed by boiling the powdered drug in distilled water, stained in safranin and mounted in glycerine.

Physico-chemical

The air-dried seeds were coarsely powdered to 10/44 (sieve size) and subjected to the determination

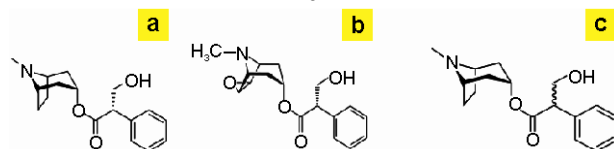


Fig. 1—Chemical structures of Hyoscyamine (a); Scopolamine (b) and Atropine (c)

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of moisture content (loss on drying at 105°C), total ash, water-soluble ash, acid-insoluble ash, extractability in different solvents. Physico-chemical constants, thin-layer chromatography (TLC) and ultraviolet (UV) aspects of the mother tincture were done following official methods⁹. The mother tincture was prepared as per Homoeopathic Pharmacopoeia of India (HPI). 100 g of coarse powder of the drug was suspended in 521 mL of 95% alcohol and 500 mL of purified water for 24 h at room temperature. It was filtered and made up to 1,000 mL using the same solvent ratio. Percolation method was used for the preparation of the mother tincture⁹.

HPTLC analysis

The mother tincture was used for the high-performance thin-layer chromatography (HPTLC) study. The extract was spotted in the form of band of 4 mm width with a Camag microliter syringe on a precoated silica gel aluminum plate 60F-254, (5×10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom Enterprises, Mumbai). A constant application rate of 6 mL/sec was employed. The slit dimension was kept at 4×0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of toluene: ethyl acetate: methanol: ammonia mixed in the volume ratio of (9:9:1.5:0.3) and 10 mL of mobile phase was used for chromatography. Linear ascending development was carried out in a 10×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature for 20 minutes. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help

of a hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by winCATS software (v 4.05, Camag) resident in the system¹⁰⁻¹².

Results

Macroscopy

Seeds reniform 3-4 mm broad and upto 2.5 mm wide, greyish black, bilaterally compressed with furrow having hilum at centre. Surface is undulated with ridges and furrows and testa reticulated and finely pitted (Plate 1a).

Microscopy

The testa consists of outermost epidermis with prominent 1-layered malphigian cells, which possess lignified, striated radial walls (Plate 1b, 2 a, b). The epidermis is covered by a thick cuticle which is often marked with sharp projections or papillations (Plate 2c, d). Beneath the epidermis, the hypodermis consists of a narrow layer of nucellus made of elongated slightly thick walled cells, in few with dense contents. The nucellus is followed by a layer of inner epidermis (Plate 1b & 2a, b). The malphigian cells become enlarged towards the hilum (Plate 2d). The nucellus also becomes precocious at the hilar end with some large cells filled with dense contents. Tracheary bundles also occur in the nucellus near the hilum (Plate 2 c, d).

The seed endosperm consists of 1-layered epidermis made up of narrow elongated cells (Plate 1b, 2 a, b) followed by endosperm made up of large polygonal to spherical, thin walled cells containing starch grains and oily

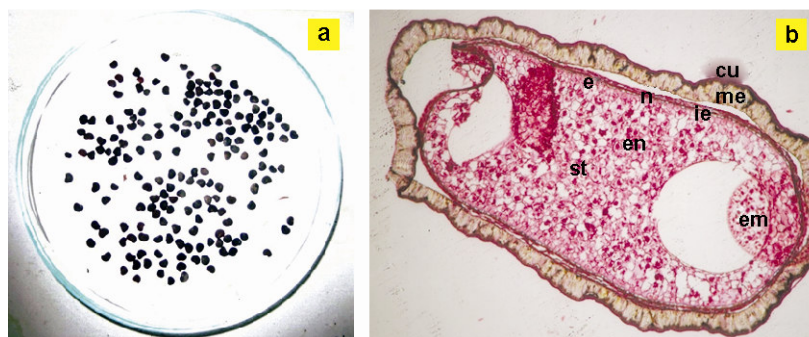


Plate 1—a. Dried seeds, b. T.S. of seed (Abbreviations: cu – cuticle; me – malphigian epidermis; ie – inner epidermis; en – endosperm; em – embryo; st – starch)

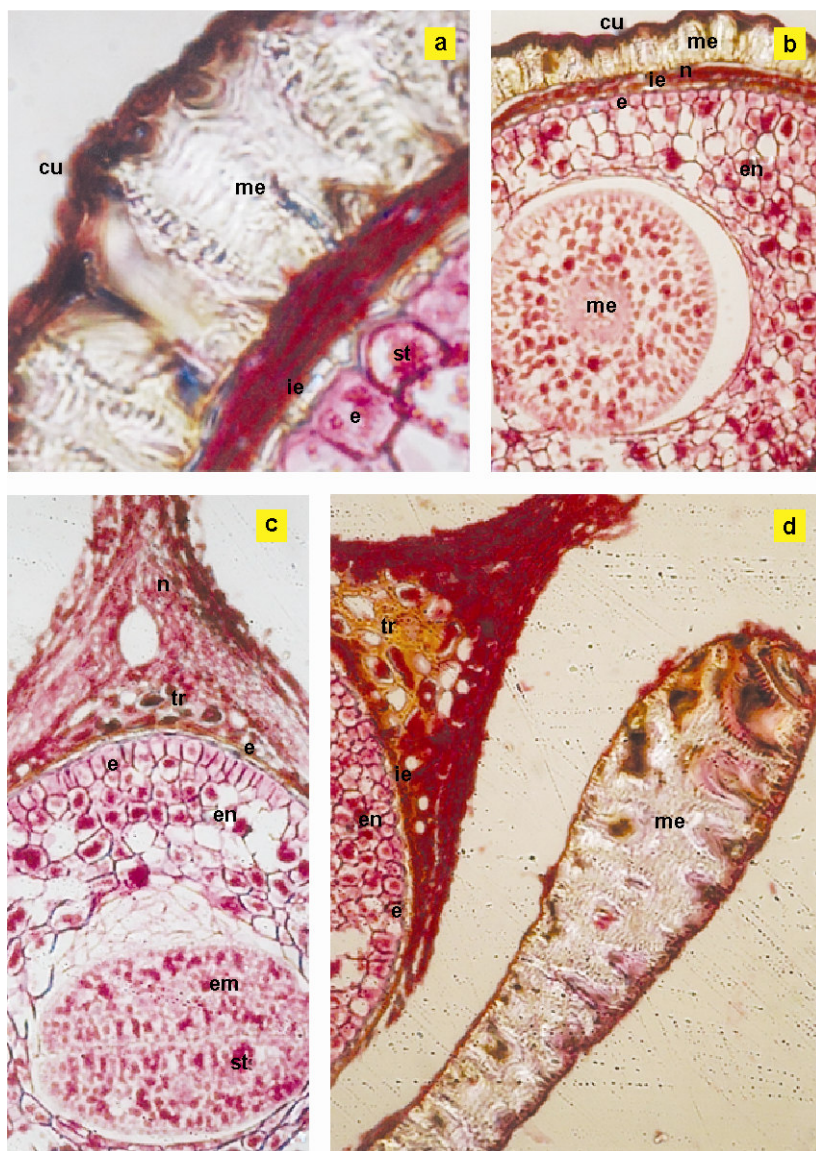


Plate 2—a. T.S of seed coat (enlarged) X 265; b. T.S. of seed with endosperm and embryo X 98; c. L.S. of seed at hilar end X 102; d. L.S. of seed with nucellus at hilar end X 108 (Abbreviations: cu – cuticle; me – malpighian epidermis; n – nucellus; ie – inner epidermis; e – epidermis of endosperm; em – embryo; en – endosperm; tr – tracheary bundle; st – starch)

matter (Plate 1b, 2b, c). The embryonic tissue inside the endosperm is made of dense contents filled with starch and oily substances (Plate 2 b, c).

Powder microscopy

The powder microscopy showed presence of several pieces of testa in surface showing polygonal malpighian cells. Malpighian cells contain with underlying endosperm. Pieces of endosperm tissue possess starch grains and oily globules. Pieces of brownish fragments of nucellus tissue and starch grains either simple or compound of various sizes were also observed

Organoleptic characters

The powder colour was observed to be brownish black with white speckles; coarse in touch, strongly pungent and taste without any characteristics.

Physico-chemical studies

The determined data under the physico-chemical study for the raw drug is summarized in Table 1 and that of the mother tincture preparation and its standardization in Table 2 and 3, respectively. Results of physico-chemical studies are summarized in Tables 1-3 are substantive for authenticating and distinguishing from adulterants.

HPTLC finger printing

The profile of the chromatographic separation scanned at 254 nm, reveals eight spots (Fig. 2 and 3) of which spots 7, 6 and 5 possess maximum composition with R_f at 0.56, 0.51 and 0.42, respectively. On the other hand, chromatogram scanned at 366 nm revealed six spots (Fig. 4 and 5) with spots 1, 2 and 3 showing maximum composition at R_f 0.16, 0.32 and 0.40, respectively. It is evident from the data that these are

characteristic for the studied drug, which will help in identification and authentication of the mother tincture. These are considered as valuable standards in pharmacopoeia. At 254 nm, eight spots appeared at R_f 0.19, 0.22, 0.25, 0.33, 0.42, 0.51, 0.56 and 0.65 (Fig.2 and 3) with various concentrations whereas at 366 nm, six spots

Table 1—Standardization of raw drug

S. No.	Parameters	Quantitative values (±) (% w/w)
1.	Moisture content (loss on drying at 105° C)	5.89
2.	Total ash	2.29
3.	Acid insoluble ash	0.27
4.	Water soluble ash	0.62
5.	Alcohol soluble extractive	7.67
6.	Water soluble extractive	8.72
7.	Extractive values in	
	a. Hexane	22.62
	b. Chloroform	26.52
	c. Methanol	8.80

Table 2—Formulation of mother tincture (percolation technique used)

Alcohol	50 % v/v (based on MEV)
Drug strength	1/10
Preparation :	
<i>Datura ferox</i> in coarse powder	100 g
Strong alcohol	521 mL
Purified water	500 mL
To make 1,000 mL of the mother tincture	

Table 3—Standardization of the mother tincture

S No	Parameters	Observations
1.	Organoleptic profile	
	Appearance	Hazy
	Color	Brown
	Odour	Pleasant and aromatic
2.	Sediments	Absent
3.	Weight per mL	Not more than 0.92 g
4.	Total solids	Not less than 0.46 % v/v
5.	Alcohol content	46-49 % v/v
6.	pH	6.58
7.	λ max	382 nm

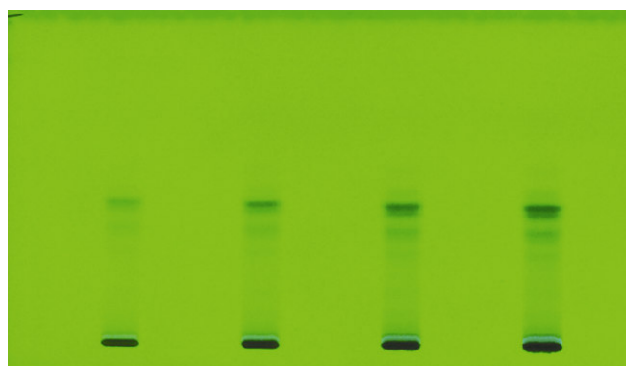


Fig. 2—High Performance Thin Layer Chromatography (HPTLC) finger printing (toluene: ethyl acetate: methanol: ammonia 25 % (9:9:4.5:0.3) v/v of *Datura ferox* mother tincture scanned at 254 nm.

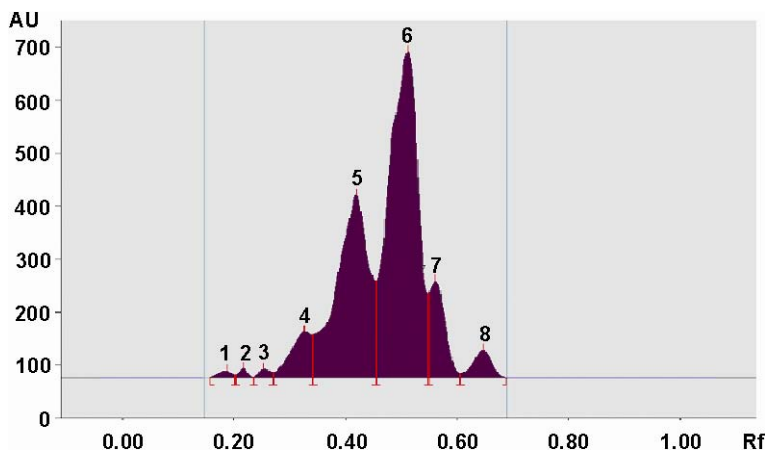


Fig. 3—High Performance Thin Layer Chromatography (HPTLC)- Chromatogram (toluene: ethyl acetate: methanol: ammonia 25%) (9:9:4.5:0.3) v/v of *Datura ferox* mother tincture scanned at 254 nm.

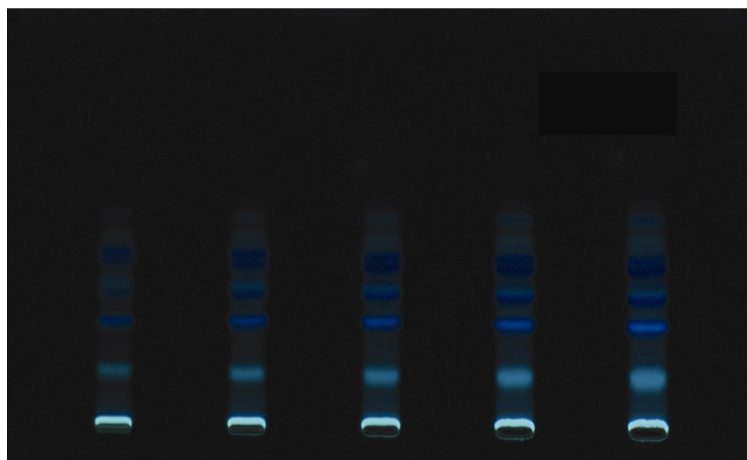


Fig. 4—High Performance Thin Layer Chromatography (HPTLC) finger printing (toluene: ethyl acetate: methanol: ammonia 25%) (9:9:4.5:0.3) v/v of *Datura ferox* mother tincture scanned at 366 nm.

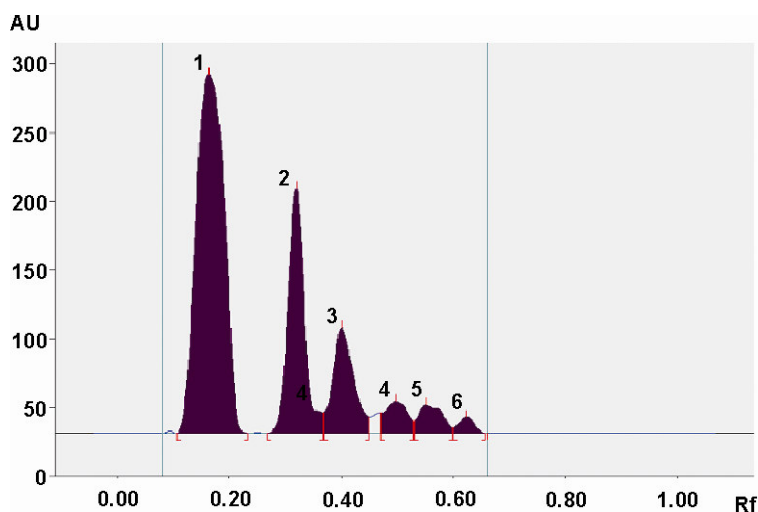


Fig. 5—High Performance Thin Layer Chromatography (HPTLC) chromatogram (toluene: ethyl acetate: methanol: ammonia 25%) (9:9:4.5:0.3) v/v of *Datura ferox* mother tincture scanned at 366 nm.

appeared at R_f 0.16, 0.32, 0.40, 0.50, 0.55 and 0.63 (Fig. 4 and 5).

Discussion

In transection, the outermost epidermis of testa consists of a conspicuous lignified or corky malphigian layer covered by a thick cuticle. The malphigian cells become enlarged near the hilum. The mesophyll in the seeds of *Datura* sp. was reported thin walled, rectangular or sub undulate cells with reticulate thickenings while presently the nuceller hypodermis is made of elongated cells with reticulate thickenings as observed earlier¹². Further, the nucellus is precocious at the hilar end. Vascular bundles near the chalaza were reported in *Datura* sp. which is presently confirmed¹². The seed endosperm and

embryonic tissue is densely filled with starch grains and oily depositions.

Conclusion

The powder microscopic features and organoleptic characters along with the anatomical and physico-chemical studies including HPTLC fingerprints are diagnostic to establish the pharmacopoeial standards for the drug.

Acknowledgements

Dr. R. K. Manchanda, Director General, Central Council for Research in Homoeopathy, New Delhi, for the facilities and encouragement, Dr. Uday Kumar, Deputy Director, Pathology Division, National Institute of Nutrition, Hyderabad for the

microtome facility and T. Sheshashena Reddy for some assistance are acknowledged.

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