A CONTRIBUTION TO THE CHEMICAL STUDY OF DATURA FEROX L. GROWN IN EGYPT

A. HIFNY SABER, S.I. BALBAA AND ASHGAN YOUSSEF

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, United Arab Republic

Datura ferox L. was found growing wild among tomato plants in the Experimental Farm of the Faculty of Agriculture, Cairo University at Guiza. This species of *Datura* has never been previously mentioned to grow in Egypt; thus the seeds seem to have been probably brought inadvertently with tomato seeds from abroad. No work has been done on *Datura ferox* L. grown in Egypt, but with regard to the nature of the alkaloidal content of this plant growing in other parts of the world, the published works show great variation in their findings. Jose Costa[1] stated that it contains hyoscyamine and atropine, while Libizov[2] reported that it contained hyoscyamine and little hyoscine. Barnard and Finnemore,[3] however, claimed that it contains hyoscyamine as the major alkaloid.

In 1948, Evans and Partridge[4] stated that it contains hyoscine as the principal alkaloid together with some meteloidine. It seems, however, that *D. ferox* is one of the solanaceous plants which are liable to change the nature of their alkaloidal content. Hence, it was deemed necessary to study the alkaloids present in *D. ferox* L. growing in Egypt.

Material

Samples examined were collected from plants grown in the ordinary loamy soil in the Experimental Station of Medicinal Plants at Guiza, Faculty of Pharmacy, Cairo University, dried in half-shade and then reduced to powder No. 36.

The Alkaloidal Content of D. Ferox L.

The powdered leaves and flowering tops of *D. ferox* L. were assayed by extracting the alkaloids with alcohol (70%) till exhaustion; the alcohol evaporated and the residue left then extracted with hydrochloric acid (about N/10). The clear acidic solution was made alkaline with a dilute solution of ammonium hydroxide and then extracted with successive portions of chloroform. The alkaloids in the chloroform were determined in the usual way (B.P. 1958). The leaves and flowering tops were found to contain 0.25% of total alkaloids calculated as hyoscyamine.

Examination of the Total Alkaloids of D. Ferox L. Using Paper Chromatography

To the titrated liquid from the assay of total alkaloids, dilute solution of ammonium hydroxide was added and then the alkaline solution extracted with successive quantities of chloroform till exhaustion, washing each chloroformic extract with the same 10 c.c. of water. The chloroform was then distilled off; 3 c.c. of alcohol were added to the residue and then evaporated to dryness. The residue was dissolved in a suitable volume of chloroform, then suitable volumes (0.01 to 0.03 c.c.) of this chloroformic solution were applied to a Whatman filter paper No. 1. The bases were developed by using *n*-butanol-glacial acetic acid-water in the ratio of 10:1:5 respectively; the butanol layer was used as the mobile phase and the aqueous acidic layer for saturation of the atmosphere in the jar. The spots were located by spraying with Dragendorff's reagent.[5] For the identification of the spots, reference hyoscine and atropine as well as mixture of these two substances and the chloroformic solution of the residue of total alkaloids were developed simultaneously on the same chromatogram. All the experiments were carried at room temperature (about 25°C.).

Discussion.—Using this method, the chloroformic solution of the alkaloidal residue of *D. ferox* showed only two spots, A and B, which were distinctly separated (Fig. 1), the spot A being developed at the level of hyoscine (R_f 0.6) and the spot B (R_f 0.90) at a higher level than that of standard atropine (R_f 0.73). On the other hand, when this solution was mixed with the solution of reference atropine and hyoscine, the mixture thus obtained showed three spots, one at the level of both spot A and the spot of hyoscine, the second at the level of atropine while the third at the level of spot B and higher than that of atropine.

These showed that *D. ferox* contains hyoscine and another substance which is not atropine.

Separation of the Alkaloids by Column Chromatography

The alkaloidal extract of 100 g. of powdered leaves and flowering tops of *D. ferox* L. was dissol-

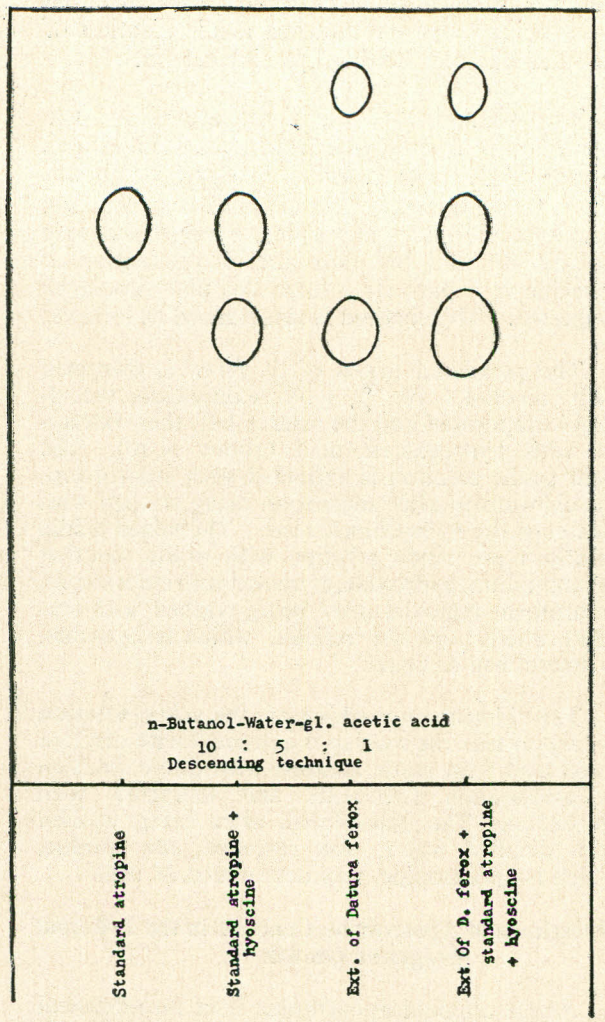

Fig. 1.—Chromatgram of alkaloidal extract of *D. ferox* hyoscine and hyoscyamine.

ved in a few millilitres of ether saturated with Sorensen's phosphate buffer[6] pH 7.3, and transferred to a chromatographic column 2 cm. in diameter containing 20 g. of purified kieselguhr, previously thoroughly mixed with 10 c.c. of the buffer. Ether saturated with the buffer was used as eluent. Substance of spot B separated first, followed by substance of spot A which separated last but the intermediate fractions were found to contain mixture of the two.

Trials to separate substance A from substance B completely from each other were carried out by changing the pH of the column. Paper chromatography has been made use of as pilot runs to discover the optimum conditions for separation, prior to using the columns. Several series of runs were made, using Whatman filter paper No.1 buffered with McIlvain citrate buffer [7] and using ether saturated with water as the running solvent. Suitable volumes of standard 1% chloroform solution of atropine and of standard 1:1 hyoscine: atropine mixture as well as of the chloroformic solution of the alkaloids of *D. ferox* L. were applied to the filter papers with different pH and results compiled in Table 1.

TABLE 1.—R_f VALUE AT DIFFERENT pH.

pH	Standard atropine	Standard hyoscine	Substance A	Substance B
2.2	0	0.04	0.04	0.80
3	0	0.06	0.06	0.83
4	0	0.09	0.09	0.85
5	0	0.10	0.10	0.92
6	0.06	0.34	0.34	0.92
7	0.12	0.67	0.67	0.93
8	0.43	0.90	0.90	0.93

From Table 1, it is obvious that the best separation by paper chromatography using ether saturated with water as the mobile phase is at pH 6. Consequently at this pH, separation of the bases present in the extract of *D. ferox* L. was tried by column chromatography.

Procedure.—Twenty g. of kieselguhr were mixed thoroughly with 10 c.c. of the citrate buffer pH 6 and packed in a column 30 cm. long with an internal diameter of 2 cm., 2 g. at a time. The residue of the purified alkaloidal extract of 100 g. of the powdered leaves and flowering tops of *D. ferox* was dissolved in 10 c.c. of ether saturated with the buffer, and transferred on to the kieselguhr column. Elution was carried out with ether saturated with the buffer; 20 fractions, 5 c.c. each, were collected or till exhaustion, then elution was continued with chloroform saturated with the buffer. The presence and chracterisation of any alkaloid in each fraction were achieved by Mayer's reagent and by paper chromatography; the spots being located by Dragendorff's reagent.

Discussion of Results.—Using the above-mentioned method, substance B was found to be eluted with ether, while substance A was found to be eluted with chloroform; thus substance A and substance B could, therefore, be successfully separated from each other.

1. Substance A was found to be the alkaloid hyoscine for the following reasons :

(*a*) It has the same R_f value as that of the standard hyoscine using *n*-butanol-glacial acetic acid-water in the ratio of 10:1:5

(*b*) It has the same R value as that of standard hyoscine when using citrate buffered papers having different pH ranging from 2.2 to 8 (see Table 1).

(*c*) Its picrate salt crystallises in the form of needle-shaped crystals which on re-crystallisation from boiling water form irregular scales of m.p. 190°C., characters identical with those of pure hyoscine.

(*d*) Its aurichloride salt crystallises in the form of needle-shaped crystals, serrated on both edges, of m.p.208 to 209°C.,identical with that of hyoscine aurichloride.

II. Substance B proved to be the alkaloid apoatropine for the following reasons :

(*a*) The picrate, when examined, appears as small yellow needles having the m.p. 170°C. nearly similar to that of apoatropine picrate (m.p. 168°C. as mentioned by Manske).[8]

(*b*) Its aurichloride crystallises in the form of fine yellow needles, m.p. 112°C., identical with that of apoatropine aurichloride.

It may, therefore, be concluded that *D. ferox* L. grown in Egypt contains the alkaloids hyoscine and apoatropine.

Hyoscyamine and atropine were not found in *D. ferox* grown in Egypt. Furthermore, meteloidine found by Evans and Partridge[4] to be present in *D. ferox* grown in England (picrate in nodules, m.p. 177°C.; aurichloride, in needles, m.p. 149°C.) could not be detected in the plant grown in Egypt.

A Simple Quick Method for Separation of Apoatropine from Hyoscine

On purifying the total alkaloids of *D. ferox* before being chromatographed, trials were made to remove the impurities present, e.g., resin and chlorophyll, as much as possible by washing the solution of the alkaloids, after acidification with hydrochloric acid, with chloroform. But it was of peculiar interest that the chromatogram of the total alkaloids left, showed only one spot corresponding to that of hyoscine while the other spot of apoatropine did not show up, indicating its absence. Eventually the chloroformic washings, after being mixed and concentrated, were examined and found to give positive tests for alkaloids and on chromatographing showed only one spot at the level of apoatropine. This indicates that the chloroform has totally extracted the apoatropine from the aqueous acidic solution of the total alkaloids leaving hyoscine behind.

This phenomenon was made use of in the separation of apoatropine from hyoscine and thus in case of *D. ferox* L. grown in Egypt which was found to contain no other alkaloids but the two, viz., hyoscine and apoatropine, a rapid and easy method for the isolation and determination of hyoscine in a pure state from this plant could be suggested. This method is summarised as follows:

The powdered organ of *D. ferox* is extracted with alcohol (70 %) till exhaustion, the alcohol evaporated and the residue left, then extracted with hydrochloric acid (about N/10). The clear acidic solution is extracted with chloroform, the chloroform after separation being washed with 10 c.c. of the hydrochloric acid. The mixed acidic solutions are made alkaline with dilute solution of ammonium hydroxide, extracted with chloroform, chloroform removed after being washed with distilled water, and the residue, which is hyoscine, is determined as usual.

The chloroform washing of the acidic solution is evaporated, the residue is extracted with distilled water and then made alkaline with dilute solution of ammonium hydroxide and extracted with chloroform. The chloroform, after being washed with distilled water, was removed, the residue, which is apoatropine, was determined as usual.

Hyoscine and Apoatropine Contents in the Different Stages of Growth

As the alkaloid apoatropine is of no medicinal value, the activity of the plant is, therefore, mainly due to hyoscine; thus it was found necessary to follow the development of each of these two alkaloids during the life history of the plant and to find out the stage at which the plant contains the maximum of hyoscine content and the minimum of apoatropine content. Samples of the seeds, seedlings and plants at different stages of growth as well as flowering and partly fruiting tops were assayed for the total alkaloidal content and for hyoscine and apoatropine content and the results were compiled in Table 2.

Table 2 shows that the seeds contain hyoscine only, while apoatropine is totally absent.

On germination, apoatropine becomes the principal alkaloid in the seedlings, reaching 0.13%, while the hyoscine content drops to 0.03%. As the seedlings grow older, the percentage of apoatropine decreases while that of hyoscine increases.

TABLE 2.—HYOSCINE AND APOATROPINE IN *D. ferox* AT DIFFERENT STAGES OF GROWTH.

Percentage of	Seeds	Seedling 5 day-old	Whole plant without root	
			18-day old	35-day old
Hyoscine ..	0.100	0.030	0.190	0.190
Apoatropine	—	0.130	0.080	0.060
Total ..	0.100	0.165	0.280	0.257

TABLE 3.—HYOSCINE AND APOATROPINE IN FLOWERING TOPS OF PLANTS AT DIFFERENT STAGES OF GROWTH.

Percentage of	Flowering tops 50-day old plant	Flowering and fruiting tops	
		Plants 65-day old	Plants 90-day old
Hyoscine	0.160	0.190	0.100
Apoatropine	0.050	0.060	0.080
Total	0.210	0.250	0.180

TABLE 4.—PERCENTAGE OF HYOSCINE AND APOATROPINE IN DIFFERENT ORGANS OF *Datura Ferox*.

Percentage of	Fl. tops	Stem	Leaf	Flower	Pericarp
Hyoscine ..	0.19	0.12	0.11	0.24	0.1
Apoatropine ..	0.06	0.03	0.09	0.07	0.07
Total : ..	0.25	0.15	0.20	0.31	0.17

Table 3 shows that the flowering and fruiting tops contain the highest percentage of hyoscine (0.19) when the plant is 65 days old; thus this is the best time of collection.

From Table 4 it is clear that the stem contains the least percentage of apoatropine, while the pericarp and leaf contain the highest percentage of apatropine in relation to hyoscine. The flower and flowering tops contain apoatropine in a percentage less than one fourth the percentage of total alkaloids.

Summary

1. *D. ferox L.* is reported for the first time to grow in Egypt.

2. Paper chromatography was applied to the total alkaloids of *D. ferox* and two distinct spots appeared on the chromatogram, one corresponding to hyoscine (Rf 0.6) and the other higher (Rf 0.9) than that of atropine (Rf 0.73).

3. Using a column of kieselguhr buffered with McIlvain citrate buffer pH 6, only two alkaloids were separately eluted, one by ether (R_f 0.9) and the other by chloroform (Rf 0.6). The latter is verified to be hyoscine while the other is apoatropine.

4. The atropine, hyoscyamine or mateloidine which are reported in the literature to be present in *D. ferox*, are not detectable by any means and in any stage of development of the plant.

5. A new rapid easy method based on the solubility of apoatropine hydrochloride in chloroform and the insolubility of hyoscine hydrochloride is suggested for the separation and assaying of these two alkaloids in *D. ferox* or when mixed together.

6. The seeds contain only hyoscine (0.1%) and no apoatropine, but on germination apoatropine becomes the principal alkaloid in seedlings of 5 days old (0.13%) while hyoscine drops to 0.03%. As the seedlings grow older, the percentage of apoatropine decreases gradually, while the percentage of hyoscine increases till the plant is mature.

7. In the mature plants, the stems contain the least amount of apoatropine (0.03%), while the pericarp and leaves contain the highest percentage of this alkaloid in relation to hyoscine.

8. Although the flowers contain the highest percentage of hyoscine (0.24 %), it is for commercial purposes that the flowering tops of *D. ferox* grown in Egypt are to be collected (percentage of hyoscine, 0.19) to constitute the drug and for the preparation of hyoscine.

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