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Detection of the presence of josciamine and scopolamine in some species of *Datura* L.

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Riassunto. *Individuazione della presenza di josciamina e scolopamina in alcune specie di* Datura *L.*
La diffusione a scopo ornamentale di specie appartenenti al genere *Datura* L. nei giardini è spesso causa di avvelenamenti occasionali, in quanto non sempre è conosciuta la tossicità di queste piante. In questo lavoro è stata condotta una indagine qualitativa, utilizzando la tecnica della cromatografia su strato sottile (TLC) per individuare la presenza dei due alcaloidi maggiormente presenti in piante appartenenti al genere *Datura*. I risultati dimostrano che sia la tecnica di estrazione che la metodica di rilevamento sono rapidi ed efficaci. Inoltre si è evidenziato la presenza di una o più sostanze presenti in tre sole specie, che attualmente sono in fase di approfondimento per la determinazione chimica, che potrebbero essere utili per una indagine chemiotassonomica del genere.

Key words: Alkaloids, *Datura* species, Quantitative analysis, TLC

INTRODUCTION

Wild and ornamental plants are commonly present in home gardens and are the first cause of poisoning. Some of these plants are cultivated for their flowers and their toxicity is unknown by people.

One of the most common cultivated genera in home gardens is *Datura*, and several cases of intoxication are reported yearly.

The use of species of *Datura* is ancient and medical uses are reported in several areas of the world (ALMAGBOUL *et al.*, 1985; ANIS & IQBAL, 1986; BALAGIZI & CHIFUNDERA, 1993; BELLAKHDAR *et al.*, 1991; BHATTARAI, 1993; CHHABRA & UISAO, 1991; DE FEO, 1992; LITZNGER, 1994; MANANDHAR, 1990; MARTINEZ, 1959; SINGH, 1995; SINGH *et al.*, 1989; VELASCO *et al.*, 1995).

The cultivated *Datura* species were introduced in Europe from South America, probably in the 16th century. Some authors refer to

genus *Brugmansia* the arborescent species belonging to *Datura*.

Wild *Datura* species were used by witches to prepare the infusions to reach an hallucinogenic status and one of the reported effects was the impresssion to fly.

We carried out a chemical test to detect the presence of well-known alkaloids of *Datura*, atropine (l-josciamine) and scopolamine, in nine species of *Datura*, wild or commonly cultivated in Italy. All the species are present in the Naples Botanical Garden.

In Tab. 1 the morphology of the nine species of *Datura* (or *Brugmansia*) used in this work is summarised (FROHNE & PFÄDER, 1984; PIGNATTI, 1982; AA.VA., 1992 ; AVERY, *et al.*, 1959).

This to make the botanical identification of the cause of intoxication easier

MATERIALS AND METHODS

Leaves of 9 species of Datura were collected in the late spring of 1999 from plants cultivated in the Botanical Garden of Naples. Two herbaceous and commonly spontaneous species (*D. stramonium* and *D. metel*) and seven arborescent species cultivated as ornamentals (*D. aurea, D. chlorantha, D. arborea, D. versicolor, D., sanguinea, D. rosei, D. cornigera*) were used.

After the harvesting, each sample of leaves was exsiccated in oven at 50 °C, subsequently the leaves were crushed in a mortar and extracted, using the following modifying method, because chlorophyll content can interfere with TLC alkaloids detection:

1 g of drug (crushed leaves) was shaked for 7 min with 10 ml of sulphuric acid 0.05M. Then 3.3 ml of ammonia (30% v/v) were added to the filtrate and the mixture was diluted to final volume of 10 ml with water, and 10 ml of ethylic ether was added to the extract, shaking for 10 min. The recovered ethereal phase was dehydrated with anhydrous sodium sulphate, filtered and dried with gaseous nitrogen. The residual was melted in 0.25 ml of methanol. 25 µl were used for the TLC.

Tab. 1 — Botanical characterization of the nine specie of *Datura* used for the TLC analysis of alkaloid

SPECIES	MORPHOLOGY
D. stramonium L.	Plant 50-200 cm, glabrous to puberulent. Leaves 5-18(-21)x4- 15 cm, ovate to elliptical, acute, cuneate to subcordate at base. sinuate-dentate to -lobed. Calyx 30-50 mm, angled; teeth (3-)5-10 mm, unequal. Corolla 5-10 cm, infundibuliform, white or purple. Capsule (2-5~)3-5-7 x (2-)3~5 cm, ovoid, erect dehiscing regularly, densely covered with more or less equal, rather slender spines up to 15 mm, rarely smooth.
D. metel L.	Plant 50-150 cm, glabrous or subglabrous stem, leaves and calyx, 10-lobed corolla and very short spines or tubercles on the capsule, frequently cultivated for ornament, often as *flore pleno* with a white, yellow or purple corolla
B. arborea L.	Small, pubescent tree up to 3 m high, with soft-hairy, ovate leaves; the nodding, trumpet-shaped flowers not over 18 cm long, white with green nerves and recurving pointed lobes, the long green calyx spathe-like, tapering to one tip.
B. aurea Safford	Sparry shrub with brittle, woody branches; large corrugated leaves 30-40 cm long; handsome pendant flowers 15 cm long, calyx with 2 to 4 lobes, and corona a rich apricot-yellow.
B. chlorantha L.	Free-blooming shrub with broad ovate, wavy leaves, and fragrant yellow, pendulous flowers; tubular calyx with 5 teeth, corona funnel-shaped; August-October; prickly fruit.
B.cornigera Hook	Shrub 1 to 3 m high, with velvet-like stem and leaves. Leaves petiolate, ovate to acuminate, scarcely pubescent. Pendulous pale yellow or white flower, 20 cm trumpets like with veins, divided into 5 lobes
B.rosei Safford	Pubescent shrubs, with 18 cm pendulous crimson-red flower, with white base and green one-lobed calyx
B. sanguinea Ruiz & Pav.	Showy tropical shrub 4 to 5 m high, the brittle branches with clustered, long-ovate soft-hairy foliage 16 cm long, and carrying the weight of large pendulous 25 cm trumpets similar to bells, flesh pink to orange-red toward apex, with yellow veins, calyx with two or more pointed lobes
B. versicolor Safford	Large shrub or small tree 2-4 m high, with sparry, brittle branches, and big oblong-elliptic, soft pubescent leaves; showy flowers like large hanging trumpets 30 cm or more long, with spathe-like calyx; the thin constricted tube expanding to the reflexed petal-lobes, pinkish and turning apricot-peach with age, fragrant in the evening

The extracts of the exsiccated and crushed leaves were analysed using TLC in order to find the presence of atropine (josciamine) and scopolamine.

Atropine sulphate and scopolamine hydrobromide (Fluka) were used as reference standards.

Preparation of the standards: 24 mg of atropine sulphate in 9 millilitres of methanol; 7.5 mg of scopolamine hydrobromide in 10 millilitres of methanol. 25 millilitres of each standard were used for the TLC. For the mixture standards 5 mil of atropine sulphate solution and 3 ml of scopolamine hydrobromide solution to a final volume of 10 ml with methanol. 25 µl were used for the TLC.

Thin layer chromatography (TLC) has been carried out using Silica gel TLC plates on aluminium support $60F_{254}$ (RdH). For the separation of the alkaloids a mixture of toluene-ethyl acetate-diethylamine (70:20:10) was used as a solvent system for the TLC.

Detection: UV-LW lamp and Dragendorff reagent

Preparation of the Dragendorff reagent:

Solution (A): Dissolve 0.85 g basic bismuth nitrate in 10 ml glacial acetic acid and 40 ml water under heating.

Solution (B): Dissolve 8 g potassium iodide in 30 ml water.

Stock solution: (A) + (B) are mixed 1: 1.

Spray reagent: 1 ml stock solution is mixed with 2 ml glacial acetic acid and 10 ml water

Results and discussion

For the appraisal of the presence of atropine and scopolamine, we carried out a preliminary TLC, in order to establish the Rf of the two substances. Using the standards we found atropine and scopolamine Rfs, 0.27 and 0.45 respectively.

Fig. 1 shows the diagram of the TCL analysis and the results are schematically reported in tab. 2.

These results show that all the species are toxic, for the presence of one or both the alkaloids considered, except *D. chlorantha*, in which alkaloids are lacking.

Therefore the cultivation of all these species in home gardens is a possible source of poisoning, for ingestion or for simple contact, in special way if this interests the mucosae.

Moreover we found the presence of a greenish spot, after coloration with the Dragendorff reagent with Rf = 0.80, which disappears after few minutes. This spot is present only in three of the species analysed (*D. arborea*, *D. versicolor* and *D. sanguinea*). In this regards, it indicates the presence of one or more substances that it is possible to use in order to characterize *Datura* species on the basis of their chemical constituents, and as an aid to understand hybrid origins.

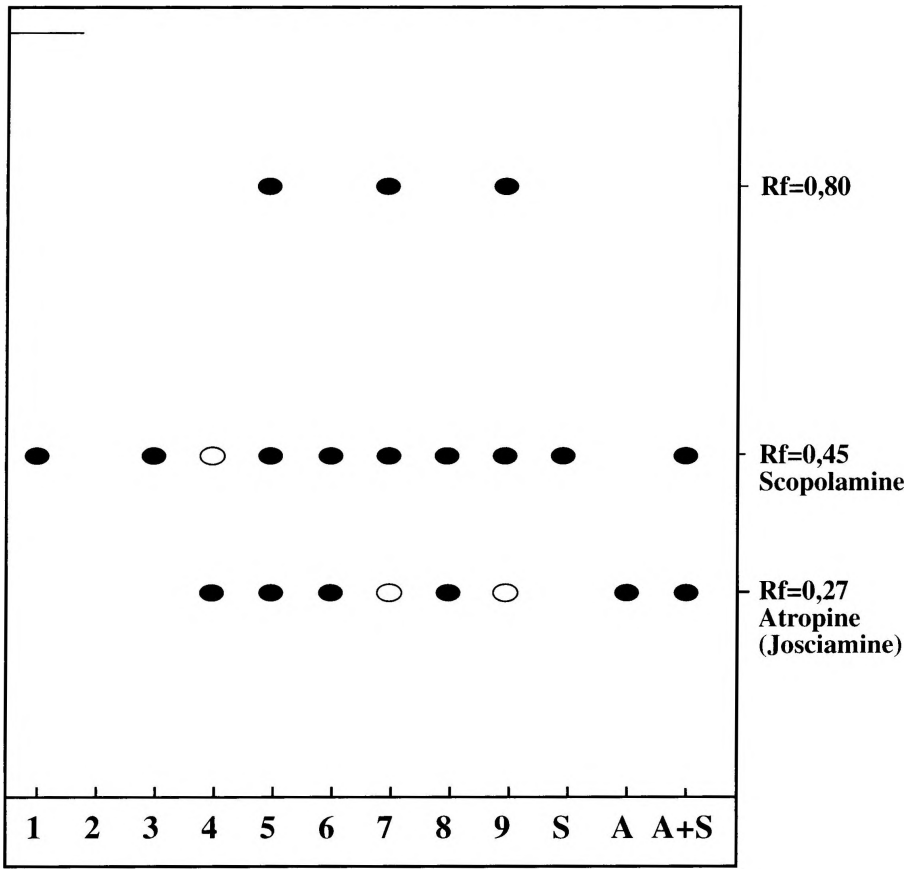

Fig. 1 – Diagram of TLC results. (1- *D. aurea*, 2- *D. chlorantha*, 3- *D. cornigera*, 4- *D. metel*, 5- *D. arborea*, 6- *D. rosei*, 7- *D. versicolor*, 8- *D. stramonium*, 9- *D. sanguinea*; A = atropine standard, S = scopolamine standard, A+S = standard misture)

Tab. 2 — The summarized results from TLC analysis

SPECIES	EXTRACTION FROM	JOSCIAMINE	SCOPOLAMINE
D. arborea	Leaves	Present	Present
D. aurea	Leaves	No	Present
D. chlorantha	Leaves	No	No
D. cornigera	Leaves	No	Present
D. metel	Leaves	Present	Traces
D. rosei	Leaves	Present	Present
D. sanguinea	Leaves	Traces	Present
D. stramonium	Seeds, Leaves	Present	Present
D. versicolor	Leaves	Traces	Present

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Abstract. *Detection of the presence of josciamine and scopolamine in some species of* Datura *L.*

The presence of *Datura* species in home gardens is often cause of occasional poisonings, because often the toxicity of these plants is not well known. In this work we carried out a qualitative analysis, using Thin Layer Chromatography technique (TLC) in order to characterize the presence of the two alkaloids mainly present in *Datura* plants. The results demonstrate that the extraction technique and the analytical method of survey is fast and efficacious. Moreover we found the presence of one or more substances in only three species, currently in further chemical determination, which could be useful for chemiotaxonomical survey.

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