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A contribution to *Apium nodiflorum* (L.) Lag. (Apiaceae) pharmacobotany

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Abstract

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Anatomical and histochemical analyses were carried out on *Apium nodiflorum,* a plant often used in folk remedies throughout Italy as a diuretic and anti-flogistic agent. The presence of secretory structures throughout the aerial part explains the use of this portion of the plant. Preliminary chemical analyses indicate the presence of flavonoids in the fruits.

Key words: Apium nodiflorum; anatomy; histochemistry; preliminary chemical screening.

Introduction

Apium nodiflorum (L.) Lag. (Apiaceae) is an entity living partially submerged in fresh water; it is found in most of Europe, but mainly in the West (Tutin 1968), as well as in North Africa, Asia, and also in North and South America as a naturalized species (Claphman et al. 1962, Cook 1974).

Folk remedies make great use of this species in many parts of Italy. A decoction, made with the aerial parts at all times of the year, is believed to have a diuretic effect and decongestive properties for kidneys and the urinary tract, as well as to be an anti-flogistic for the stomach, intestines and lungs (Chiovenda-Bensi 1960, Corsi e Pagni 1978, Pagni e Corsi 1979, Tammaro 1984).

In spite of this, the plant has never been studied either under its pharmacological or its botanical aspects. The only scientific knowledge we have is its chromosomic number of 2n=22 (Scrugli e Bocchieri 1977). Phytochemical analysis of this species has been limited to the myristicin content of the fruits in an investigation concerning both spontaneous and cultivated Umbelliferae (Harborne et al. 1969).

The present research had two aims. One was to investigate the anatomy of the plant, primarily to locate the secreting structures responsible for the production of medicinal substances in the Umbelliferae (Crowden et al. 1969, Hegnauer 1971, Fahn

1967) and to find what part of the plant is most suitable for use as drug. Histochemical techniques were mainly utilized here.

The second was to reveal any secondary products that justify the use of the species as a diuretic and an anti-flogistic based upon very preliminary screening. The latter was carried out by phytochemical methods. It focussed on flavonoids because substances responsible for diuretic and decongestive effects in the Umbelliferae (Morelli 1981, Paris et Moyse 1976) generally belong to this group.

Materials and methods

Plant were gathered from a ditch at Agnano (PI) and then cultivated in Pisa University Gardens. Exiccata in PI

Botanical analysis

For botanical and histochemical investigations hand-made sections from fresh material and sections 20 µm thick obtained from material embedded in paraffin, were processed. The following histochemical techniques were used:

Toluidine blue (Sappa 1959) as a generic cell stain	
Zinc-chlor-iodide (Jensen 1962) for cells walls	
Safranin-fast-green (Jensen 1962) for fibro-vascular bundles	
Iodine iodide tincture (Faure 1914) for starch	
α-naphthol in sulphuric acid (Faure 1914) for inulin	
Ruthenium Red (Jensen 1962) Delafield's Hematoxilin (Faure 1914)	for pectin-like substances
Feulgen (Johansen 1940) for DNA	
Phloroglucinol (Jensen 1962) for lignin	
Sudan Black (Bronner 1975) Alkanna tincture (Faure 1914)	for lipids
Sudan III followed by treatment with glacial acetic acid (Johansen 1940)	for essential oils
Fuming hydrochloric acid (Faure 1914)	for calcium oxalate
Eosin (Sappa 1959) Picric acid (Trease and Evans 1983) Millon's Reagent (Faure 1914)	for proteins

In all cases, controls were set up according to the methods suggested by their authors. Vittae were isolated from the fruit for detailed investigation by Kapoor and Kaul's method (Kapoor et Kaul 1966).

Phytochemical analysis

The Shinoda test (Shinoda 1928) was used for the flavonoid compounds. The test was performed separately on 10% infusions of the parts above-water, those underwater and the fruit, on the residue of the methanolic fruit extract; on the macerated residue from the aerial parts and fruits and their fractionations with light pretroleum and ethyl acetate.

Tests on the infusion of the underwater parts gave negative results. We, therefore, suspended analyses concerning these because they are not generally utilized in folk remedies. Moreover, this part revealed no secretory structures.

The aerial part was tested excluding the fruit because the species is used all year round even when it is not bearing fruit.

Tests on the fruit extract were repeated using hot methanol because the infusion was of a strong colour which by itself would have given a positive result. The macerate residue was fractionated by dissolving in 9 : 1 methanol/water followed by extraction with light petroleum and ethyl acetate. A red colour revealed the presence of flavonoid compounds.

Results

Botanical analyses

The plant has no secondary structure. The root (Fig. 1) shows a considerable quantity of aerenchyma in the cortical cylinder, a variable number of arches (generally 2 or 3, seldom 4 or 5) and an endodermis with Casparian thickenings. Secretory structures are absent.

The stem (Fig. 2) is hollow and the cross-section reveals numerous collenchymatic ribs in the cortical cylinder immediately under the epidermis. Thick and thin ribs alternate forming a perfect circle.

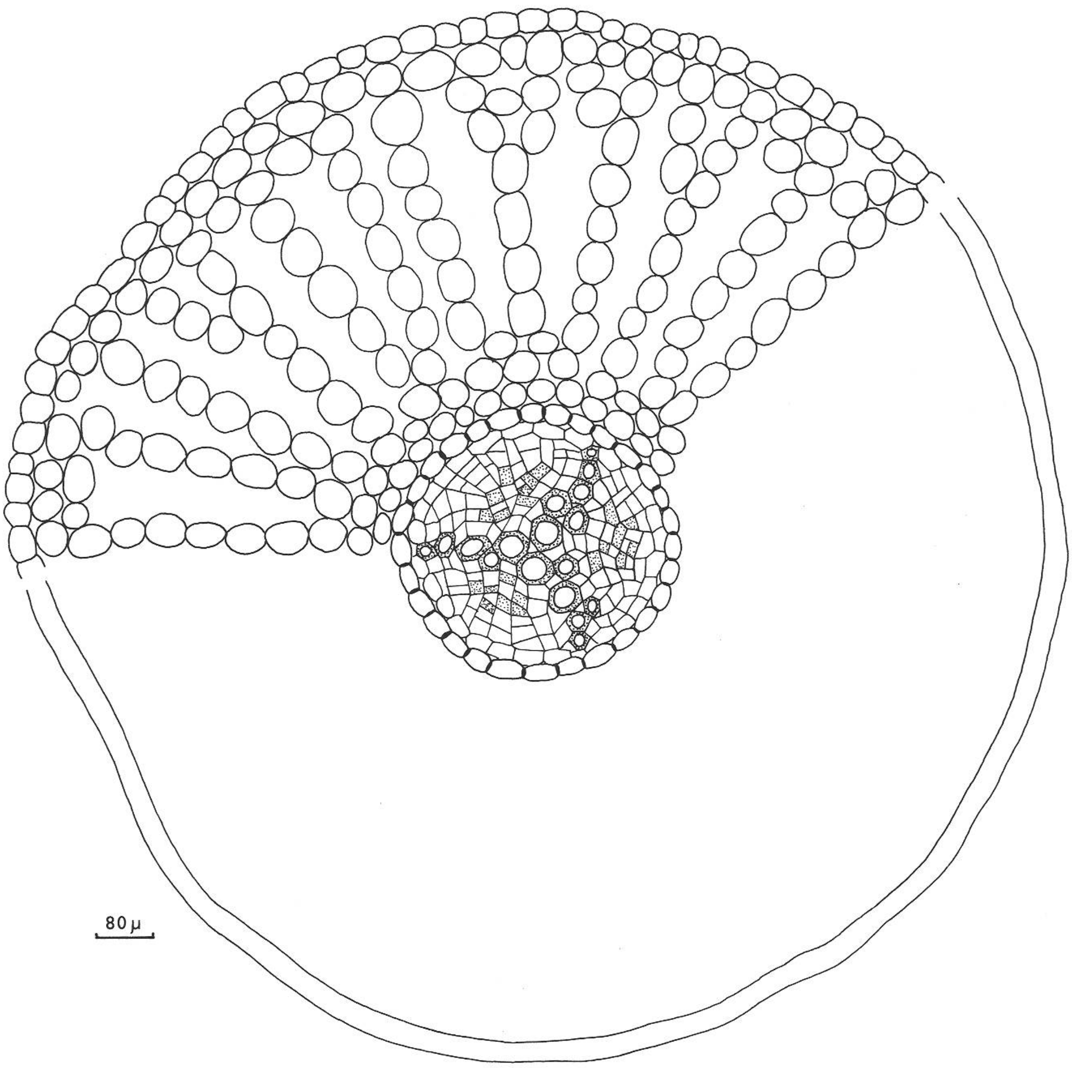

Fig. 1. Cross-section of root.

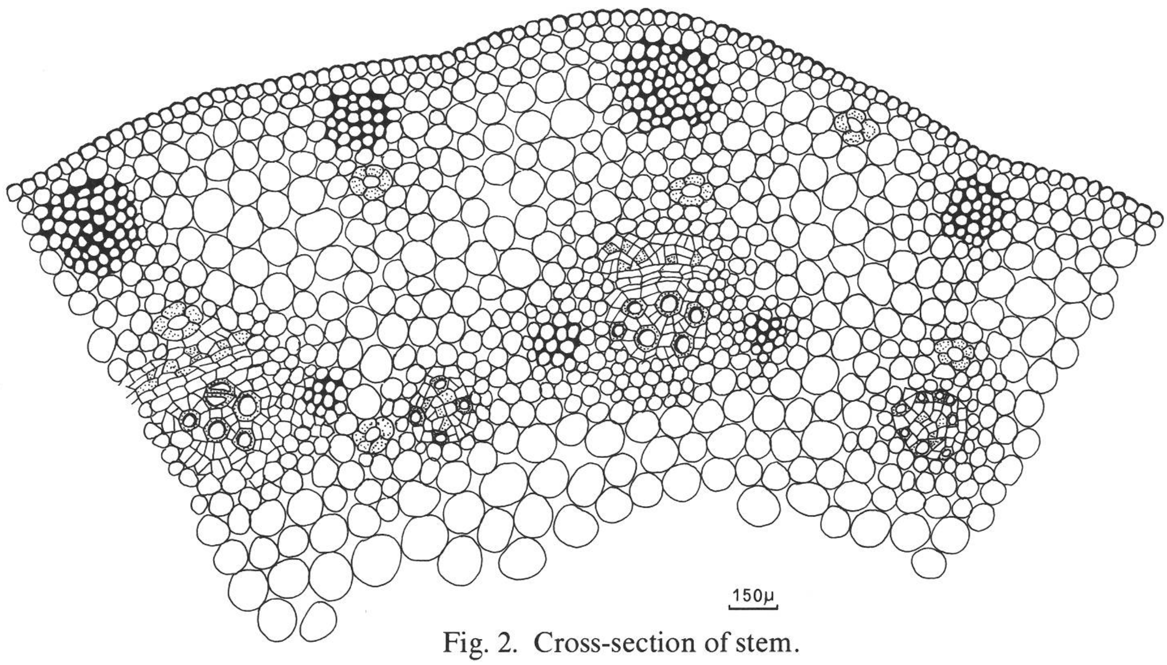

Fig. 2. Cross-section of stem.

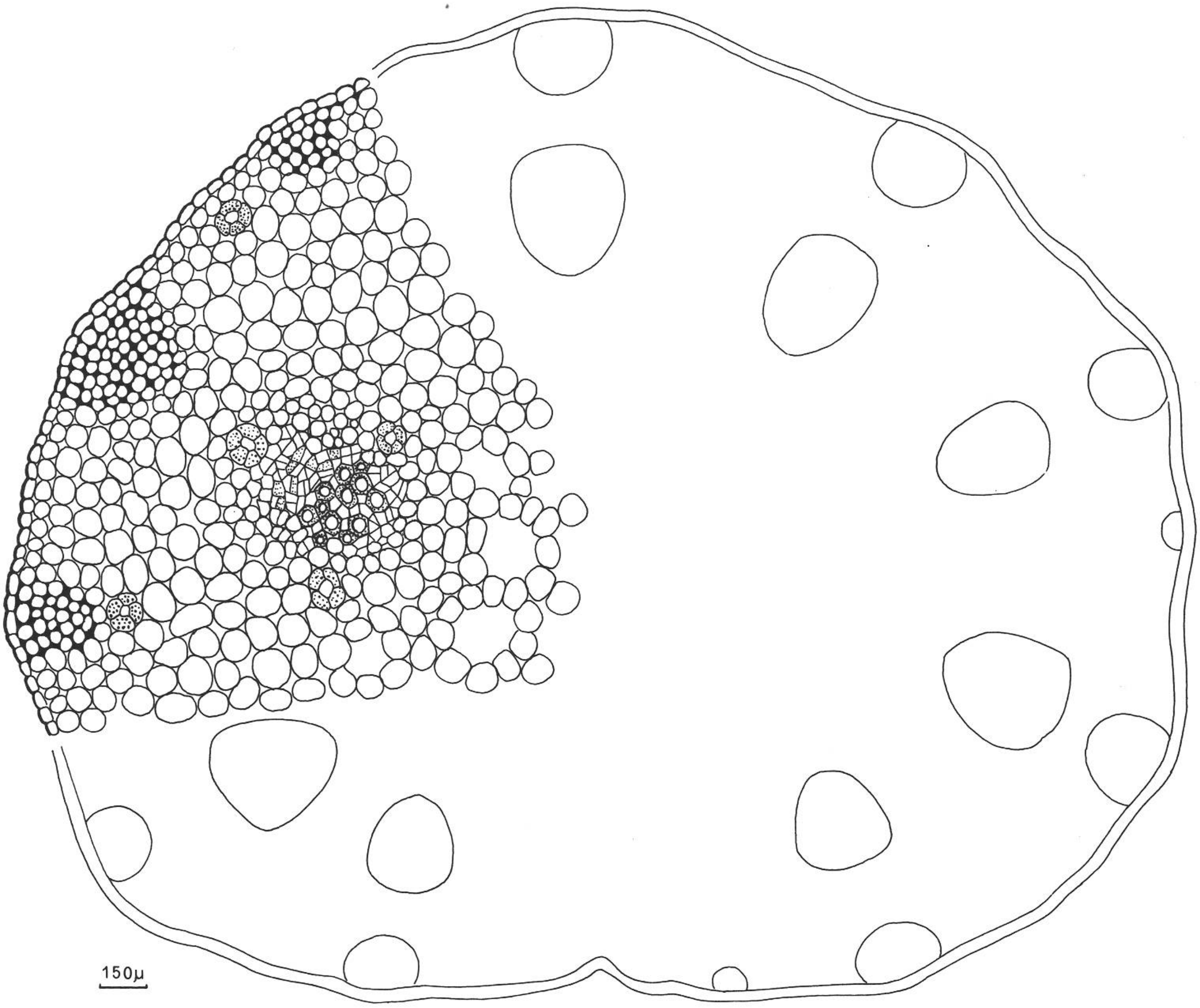

Fig. 3. Cross-section of petiole.

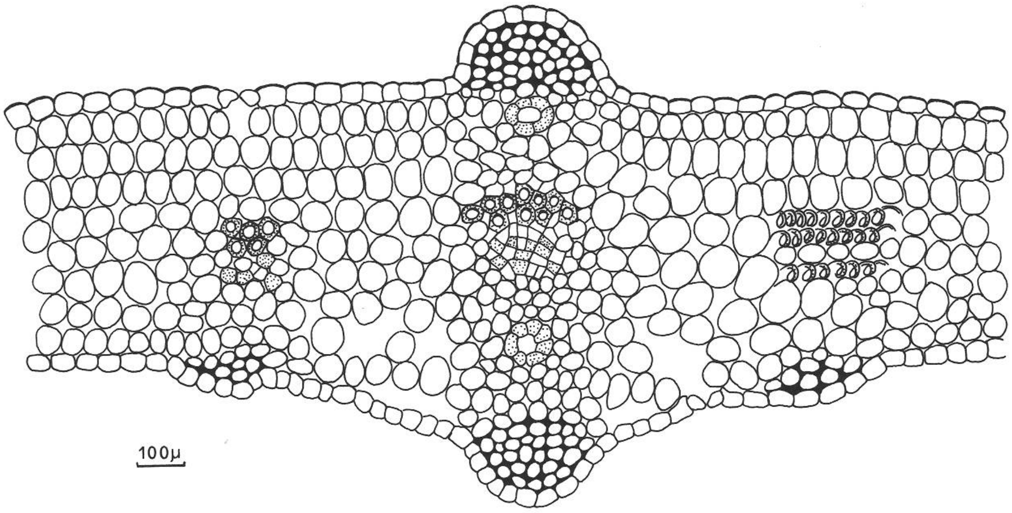

Fig. 4. Cross-section of leaf lamina.

The central cylinder shows several collateral vascular bundles, the number of which varies according to the stem size. They alternate with small concentric, external vascular bundles, only slightly lignified which could be interpreted as supernumerary bundles. All the bundles, including the smallest are surrounded by parenchymatic cells rich in starch, forming what could be considered a starch-bearing sheath.

The stem reveals many small secretory canals*. These can be observed both immediately under the epidermis and at the same level as the small and big bundles. They contain a lipophylic secretion, probably an essential oil.

The rhizome, which is short, is more evident in autumn and winter and is anatomically identical to the stem and with practically the same secretory structures.

The petiole (Fig. 3) has an aerenchyma with fibro-vascular bundles, all of the same size and forming a regular peripheral ring. They occur together with several secretory ducts whose size, arrangement and secretion are the same as in the stem.

The fibro-vascular bundles of the leaf lamina (Fig. 4) are of modest dimensions and only slightly lignified. External to each bundle is a secretory canal of reduced size.

The fruit (Fig. 5) was analysed with particular care, because it is a very important structure for diagnosis in the Umbelliferae (Davis and Heywood 1963, Tseng 1967). Furthermore, it is very frequently this part of the plant which is used as a drug from members of the Umbelliferae.

The fruit has an average length of 1.8 mm and is 0.8 mm wide (based on 20 samples). It has 5 prominent ribs and a stylopodium of identical length and width, with erecto-patent styles. The pericarp is rather thin and has 5 fibro-vascular bundles in the ribs with a considerable xylem but hardly any or no phloem. The area between each vascular bundle and the seed consists of thick cellulose-walled cells (fig. 5). This probably provides mechanical support to the fruit which is well-provided with a loose parenchyma. The seed is quite large with well-developed endosperm which consists of starchless cells rich in lipids, and, in particular, in calcium oxalate druses.

Staining revealed a layer of protein around crystals. Sometimes there is more than one crystals per cell. This indicates that the crystals are probably part of an aleurone

* On the differences between vittae and secretory canals see Corsi e Pagni (1983)

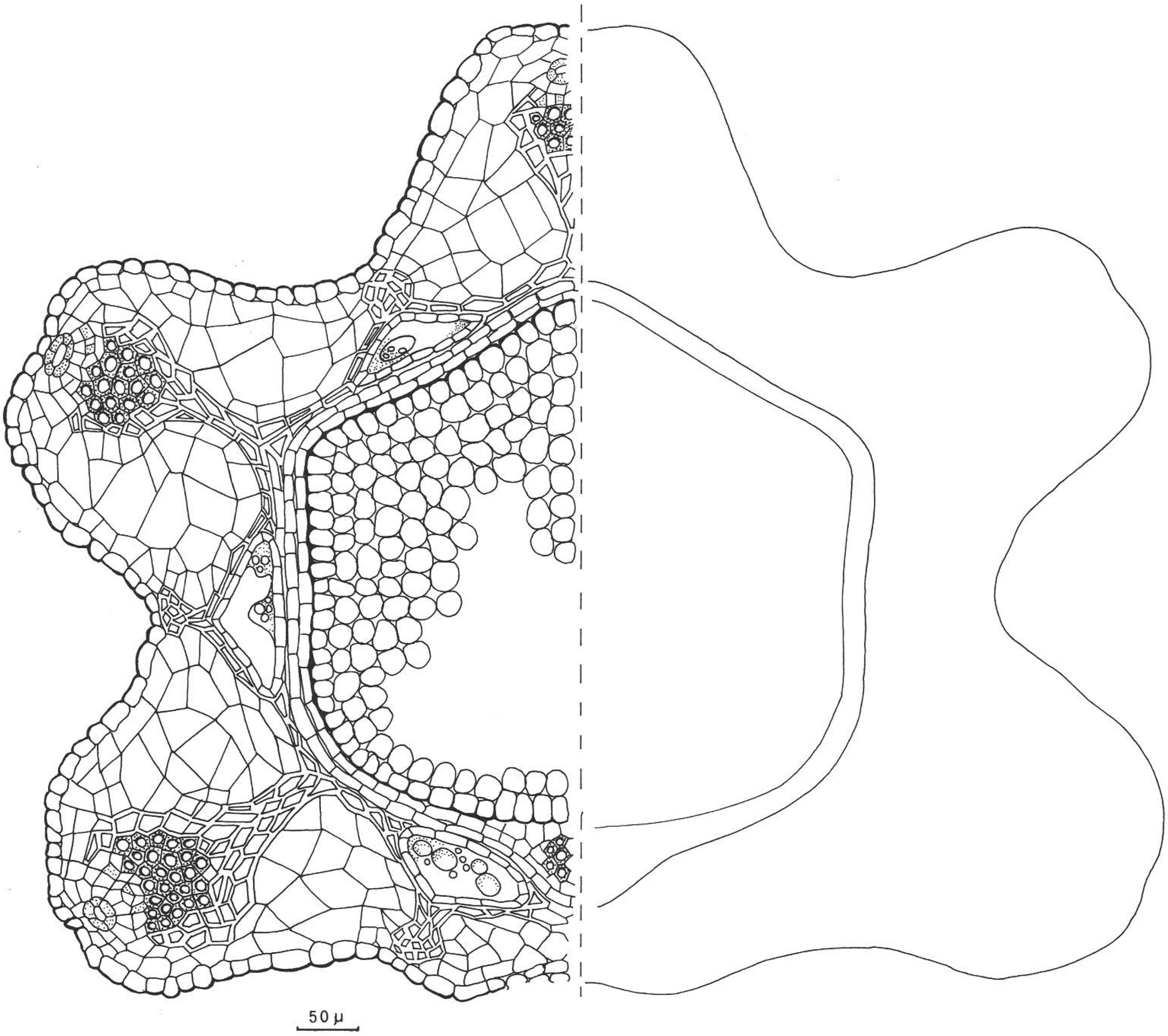

Fig. 5. Cross-section of fruit.

granule (Fig. 6). Calcium oxalate crystals, single or contained in aleurone granules, can be observed in several Umbelliferae (Spitzer and Lott 1982).

Of the anatomical features, great taxonomic value is attached to the vittae (Heywood and Dakshini 1971). They are also interesting since they appear to be site of synthesis and/or storing of biologically active secondary products (Crowden et al. 1969, Hegnauer 1971).

In the achene each vallecula has one large vitta (seldom more); its section is triangular (Fig. 7) instead of the more usual elliptical, even in the ripe fruit. Each vitta (Table 1) has an area towards the peripheral portion of the fruit which presents thick cellulose-walled cells (Fig. 5). This thickening, too, might provide mechanical support for the fruit. Specific stains reveal lipophilic secretions in the vittae (Fig. 7). This is probably an essential oil as shown for the secretory canals.

Secretory canals (Fig. 8) of reduced size can be observed on the outside of each bundle in the ribs. Their appearance is exactly like that in the vegetative organs, and they contain the same type of lipophilic secretion.

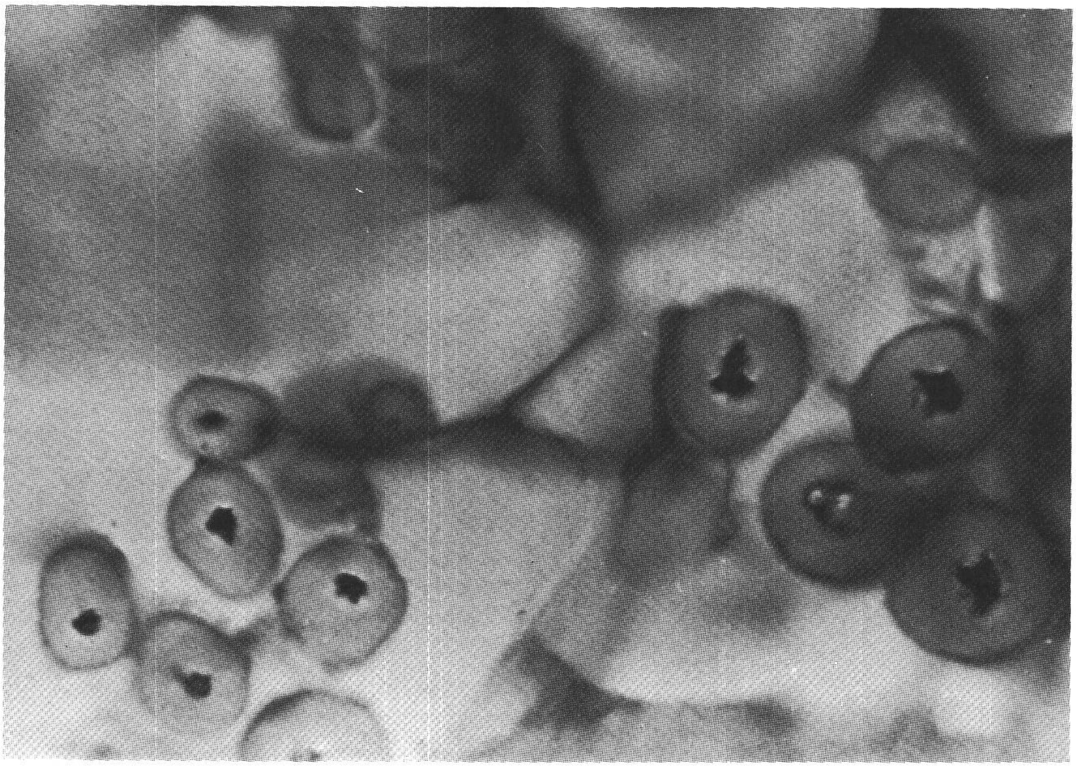

Fig. 6. Calcium oxalate druses surrounded by a protein layer (Millon's Reagent ×1800).

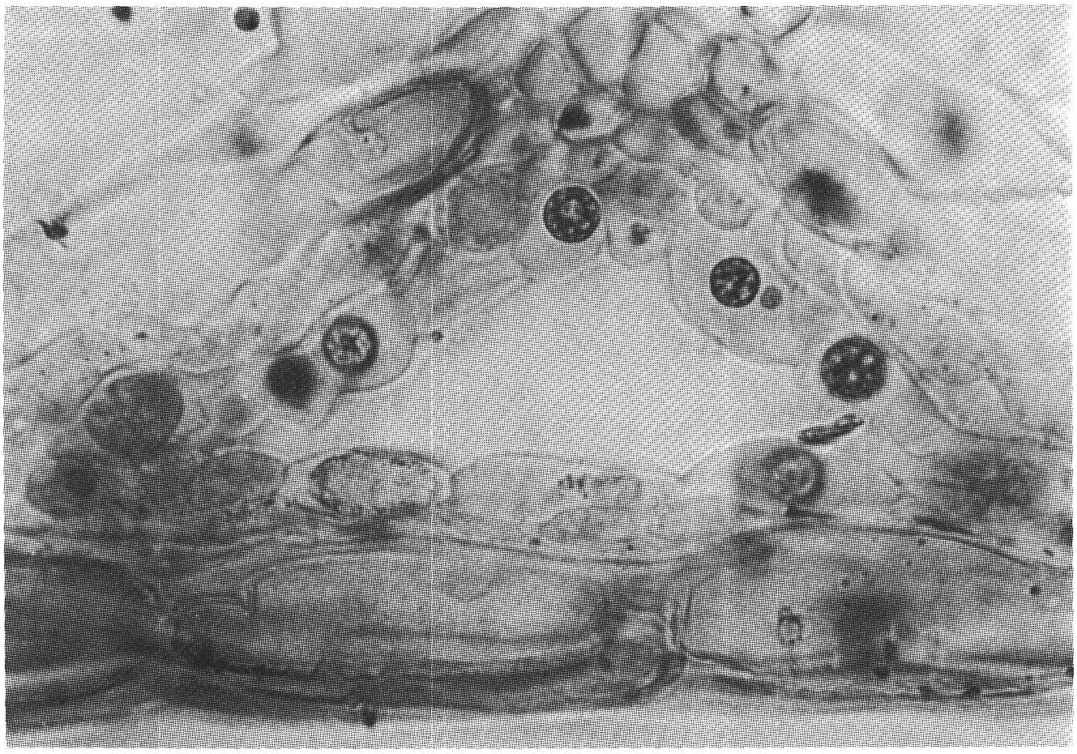

Fig. 7. Cross-section of a vitta showing lipophilic secretion in the cells (Sudan III ×1000).

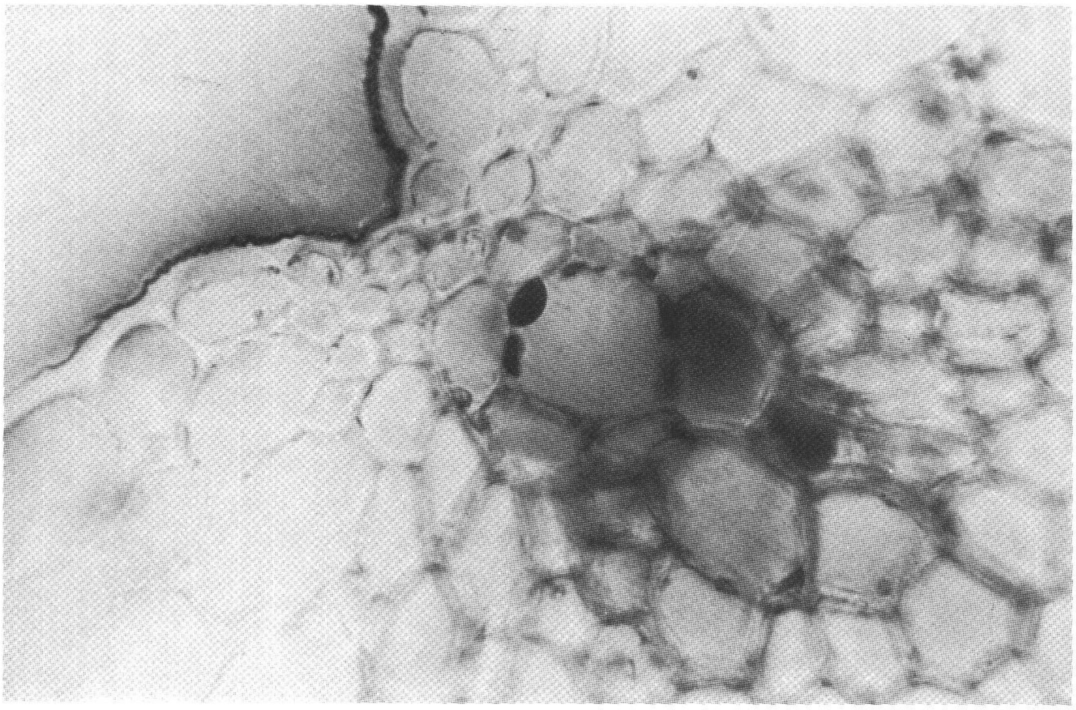

Fig. 8. Secretory canal in the fruit (Sudan III × 1000).

Table 1. Features of vittae of *Apium nodiflorum*

Position of vittae	One for each vallecula	
Isolation after treatment with KOH	Difficult to isolate even after 90 min	
Number	maximum	6
	minimum	5
	mean	5.5
Length in mm	maximum	2.6
	minimum	1.3
	mean	1.8
Width in mm	maximum	0.3
	minimum	0.1
	mean	0.2
Colour following treatment with KOH*	light brown	
Walls of cells surrounding vittae	thickened	
Number of septa	maximum	18
	minimum	10
	mean	14
Type of septa	double transversal	
Aborted vittae	–	
Anastomoses	–	

* This character is connected with the degree of thickening in vitta cell walls: a darker colour and easier isolation are related to a greater thickness in these walls.

Table 2. Preliminary test on the flavonoid compounds present in *Apium nodiflorum*

10% Infusion 15′	Aerial parts		–
	Underwater parts		–
	Fruits		–
Extraction with hot MeOH 15′	Fruits		–
Maceration with MeOH	Aerial part		+
			±
7 days	fruits		+
			±
Fractionation of maceration residue	Aerial part	light petroleum	±
		AcOEt	±
		$MeOH/H_2O$	–
	Fruits	light petroleum	+
		AcOEt	–
		$MeOH/H_2O$	–

MeOH: methanol
$MeOH/H_2O$: methanol/water 1:1
AcOEt: ethyl acetate
± Indicates a faint colouration

The features of diagnostic interest (Kapoor and Kaul 1966), investigated in vittae isolated by Kapoor and Kaul's method of maceration, are described in table 1.

Phytochemical analyses

The results of these preliminary tests on the flavonoids are summarized in table 2.

Discussion and conclusion

Apium nodiflorum has characteristics typical of underwater plants i.e. no secondary structures, massive loose parenchyma, few mechanical tissues restricted to the collenchyma, the vascular bundles of reduced size, few in number and with xylem elements only slightly lignified. The secretory structures are vittae in the fruit and secretory canals in both the fruit and vegetative organs except for the root.

Roots perhaps lack these elements because they are permanently under water and, therefore, do not require secretions to protect them from desiccation or attacks from animals. Histochemical tests revealed only lipophilic secretions in both fruit and vegetative organs. This is in contrast with results by Metcalfe and Chalk (1950) who claimed that also mucilaginous substances can be secreted in the secretory canals of the Umbelliferae. The use of entire above-water part as medicament is justified because it is here that the secretory structures are found.

It must be considered moreover that for much of the year the plant bears fruit, the vittae of which are large and contain greater quantity of secretory products than the secretory canals which are small. From the preliminary chemical analyses *A. nodiflorum* fruits seem to contain flavonoid compounds, probably in the vittae. These compounds should provide methoxyl groups which have the effect of decreasing molecular polarity. This would explain solubility in a non-polar solvent such as light petroleum. This sort of flavonoid is reported in the literature (Harborne 1971) as present in *A. graveolens,* a plant which is closely related to the species studied here.

The results of our tests do not clearly demonstrate the presence of flavonoids in the aerial vegetative organs. It must be concluded that if the decongestive and diuretic properties attributed to *A. nodiflorum* are due to flavonoids, this effect is obtained when the plant is bearing fruits. Thorough phytochemical analyses are planned to test for other chemically active substances.

The diuretic effect could also be due to the impressive amount of calcium oxalate crystals in the fruits. This may have a stimulating effect on the kidneys by irritating the kidney epithelium.

Resumé

Les auteurs ont accompli une étude anatomique et histochimique sur *Apium nodiflorum,* plante utilisée fréquemment en médecine populaire dans plusieurs régions d'Italie comme antiphlogistique et diurétique. Dans toute la partie aérienne de la plante ont été mises en évidence des structures sécretrices qui justifient son utilisation. Des tests chimiques préliminaires semblent révéler dans les fruits des composants flavonoides.

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