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Vegetative morphology and anatomy of *Cissampelos* in South Africa

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Representative samples of the four South African species of *Cissampelos* were collected for morphological and anatomical studies. Leaves and rhizomes of all four species are used in traditional medicine and any morphological and anatomical differences could be useful in forensic studies. Rhizomes of *Cissampelos* are easily identifiable because of the sieve-like appearance in transverse section of all members of the Menispermaceae, but *C. hirta* and *C. mucronata* are difficult to distinguish from one another. These two species are anatomically similar and morphologically distinguishable only by leaf texture, colour and the presence of a geniculate pulvinus in *C. hirta*. Furthermore, tanniniferous idioblasts occur in these two species but not in *C. capensis* and *C. torulosa*. Leaf size is important to distinguish *C. capensis* from the other three species and leaf shape to distinguish *C. torulosa* from the rest. *C. capensis* differs from the others in a number of anatomical features.

Introduction

The Menispermaceae comprises between 71 genera with 450 species (Kessler 1993) and 75 genera with 520 species (Watson and Dallwitz 1992 onwards) and is widespread in tropical and subtropical countries, with relatively few species in temperate regions. Approximately 25 genera with 101 species are found in Africa, of which seven genera with 13 species and two varieties are found in southern Africa (Troupin 1962, Jordaan 2000). Members of the family are mostly lianas, sometimes small trees or shrubs and occasionally perennial herbs. The leaves are alternate, petiolate, sometimes peltate and exstipulate. The lamina is simple, entire or lobed, palmately veined, often tipped with a mucro and ovate, deltoid, cordate or reniform (Troupin 1956, Botha 1975). Detailed studies of the anatomy of Menispermaceae (Maheu 1902, Solereder 1908, Metcalfe and Chalk 1965, Botha 1975, Botha and Van der Schijff 1980) have shown relative uniformity, particularly with regard to the peculiar medullary rays which separate the vascular bundles and stand out distinctly in transverse sections of the above-ground stems and rhizomes, and also with regard to the xylem which is characterised by large and conspicuous vessels. In young stems sclerenchyma fibres form arcs, which are nearly always joined by stone cells in the vascular rays to form a continuous ring.

The genus *Cissampelos* L., together with *Cocculus* Bauhin, *Stephania* Lour. and *Antizoma* Miers are southern African members of the tribe Menispermeae (Forman 1982, Kessler 1993) formerly known as the Cocculeae (Rhodes 1975). *Cissampelos* species are woody or herbaceous twining vines or lianas, or sometimes shrubs (Troupin 1960, Thulin 1993) with rhizomes (subterranean stems). The species are heteromorphic in leaf texture, pubescence and shape and are found in diverse habitats (Botha 1975, Rhodes 1975).

There are no distinct anatomical characteristics that separate *Cissampelos* from other members of the family. *Cissampelos* is an important medicinal plant genus, both internationally (as a source of curare alkaloids used traditionally as arrow poison, but also in modern medicine for its muscle relaxant properties) and locally in traditional herbal medicine.

Four species have been recorded from South Africa (Harvey 1859, Botha 1975), namely *C. capensis* L.f.; *C. hirta* Klotzsch; *C. mucronata* A. Rich. and *C. torulosa* E. Mey. ex Harv. Figure 1 shows the geographical distribution of the four South African species in the southern African region (here including Botswana and parts of Zimbabwe and Mocambique), based on herbarium specimens and the data in Troupin (1960), Troupin and Gonçalves (1973) and Botha (1975). All four species form part of the traditional *materia medica* of South Africa (Watt and Breyer-Brandwijk 1962, Hutchings *et al.* 1996, Van Wyk *et al.* 1997). Both leaves and above-ground stems and/or rhizomes are used for a variety of ailments. A detailed review of ethnobotanical uses of *Cissampelos* is in preparation (De Wet, unpublished data). A preliminary phytochemical study of *C. capensis* indicated it to be dimorphic, with a coastal form occurring on dunes and an inland form having different combinations of alkaloids.

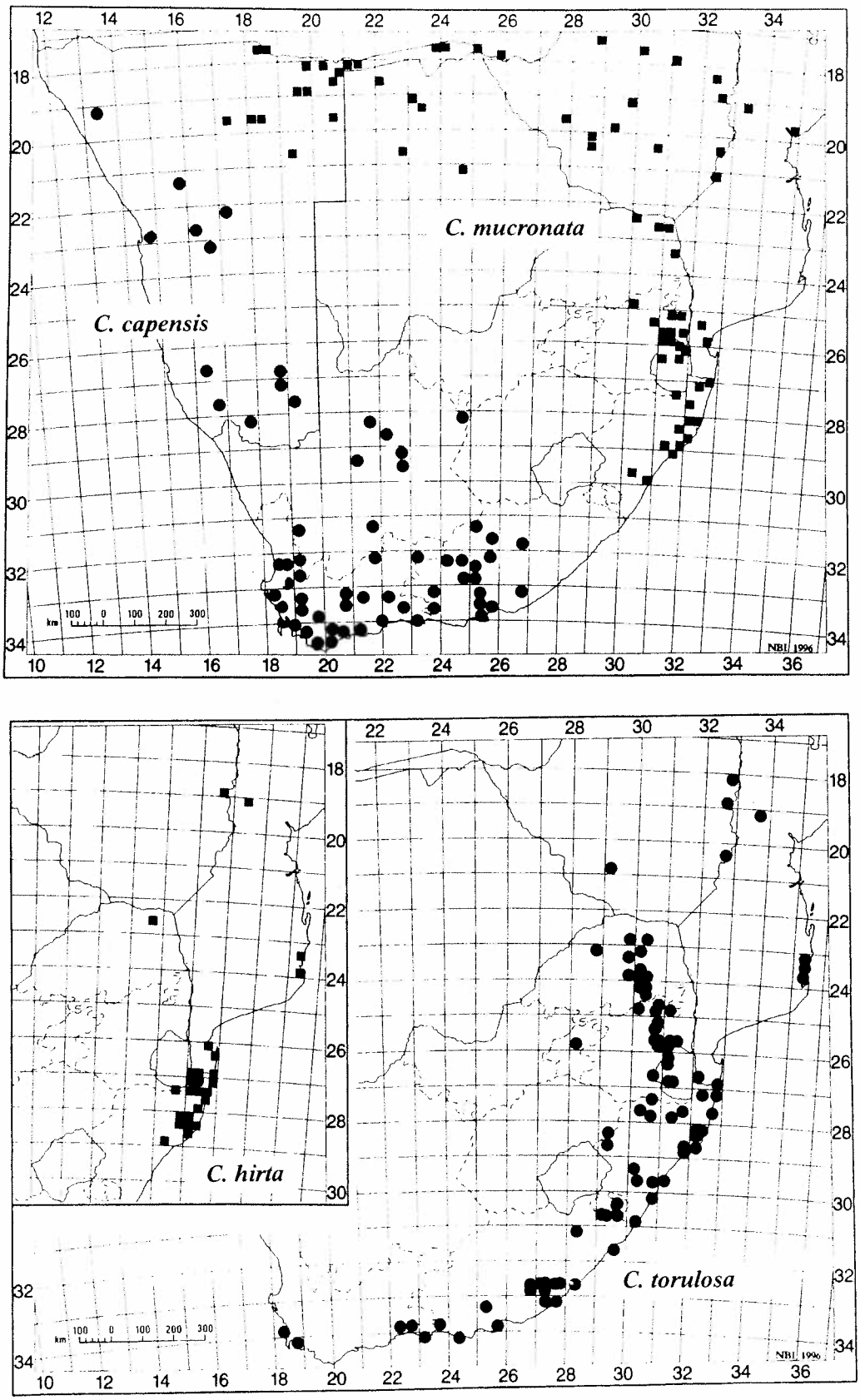

Figure 1: Known distribution of the four South African species of *Cissampelos* in the southern African region (including Botswana and parts of Zimbabwe and Mocambique)

The aim of this paper is to investigate the differences between the four South African species of *Cissampelos* and the different forms of *C. capensis* for identification purposes. A secondary aim is to characterise, from an anatomical point of view, the peculiar appearance of rhizomes in transverse section. Such information may be useful from a forensic point of view.

Materials and Methods

Voucher specimens of the material used for morphological studies were deposited in the University of Zululand Herbarium (UZ) (see Table 1). In addition, material available in the National Herbarium (PRE) was used to investigate leaf morphological variation of the four South African *Cissampelos* species. For the anatomical study, fresh leaf (blade, midrib and petiole) and stem (above-ground and subterranean) material from 13 populations of the four *Cissampelos* species was preserved in FAA. A complete list of material is given in Table 1. The material was embedded in glycol methacrylate (GMA) according to the method of Feder and O'Brien (1968). However, a minimum of 24 hours was used for the first two infiltrations in GMA and a minimum of five days for the third infiltration. The gelatine capsules containing the material and GMA were placed in an oven at 60°C for 24h to polymerise. Sections, 3–5mm thick, were cut using a Porter Blum MT-1 ultramicrotome. The Periodic Acid — Schiff/Toluidine Blue (PAS/TB) staining method was used and photographs taken using a Leitz Wetzlar microscope and AFGA (APX 25) film.

Results

Morphology

The rhizomes and leaves of the four South Africa species of *Cissampelos* are described in Table 2 and the variation shown in Figures 2 and 3 respectively. Diagnostic differences are highlighted below.

The rhizome of *C. capensis* is long and of even thickness, occasionally branched, dark brown in colour and about 10mm to 50mm in diameter. In contrast to the other species it has a rather smooth surface and no external longitudinal ridges. A transverse section reveals a distinct porous nature of the xylem, with the broad medullary rays giving the spoke-like appearance (Figure 2) characteristic of all four species. In comparison to *C. capensis* the rhizome of *C. hirta* is thinner (±10–15mm in diameter) and its surface is somewhat fluted with irregular longitudinal ridges (Figure 2B). Internally and externally the rhizome of *C. mucronata* resembles that of *C. hirta* (Figure 2C). The rhizome of *C. torulosa* is much smaller than that of the other species and has an irregular shape, abruptly tapering at the base and less fluted (Figure 2D). However, internally there is no difference in appearance.

The leaves of *C. capensis* are much smaller than those of the other three species, especially in the inland form (Figure 3A), with a typical greyish green colour and a coriaceous texture. Leaves of *C. hirta* are deltoid to cordiform (Figure 3B), with a shiny green adaxial side and a coriaceous texture. *C. mucronata* has leaves which are broadly ovate to cordate (Figure 3C), pale green and velvety and those of *C. torulosa* are reniform to deltoid (Figure 3D), dark green and papery.

Anatomy

Since all four species treated in this paper are very similar, a single description is given.

There is no clear macroscopic demarcation between rhizome and roots. The anatomy of the rhizome is similar to that of the above-ground stem, which is described below. Xylem tissue is extensive and includes very large vessels. The medullary rays are very broad with vertical rows of regularly packed parenchyma cells, which contain bell-shaped starch grains. No tanniniferous idioblasts were observed, but conspicuous secretory cells (Figures 4B, C) are present in the medullary rays of *C. hirta* and *C. mucronata*.

The outline is round with a smooth to undulate surface. Bicellular hairs, with a short basal cell and a long terminal cell provided with a lumen are present. A thick cuticle is usually visible, except in *C. torulosa* (Figure 5D) where it is very thin. Stomata occur in young stems. The epidermal cells vary from periclinally elongated to square in shape with strongly cutinised or sometimes in *C. hirta* and *C. capensis* gelatinous cell walls. The outer periclinal cell walls are

Table 1: Voucher specimens of the leaf, above-ground stem and rhizome material of the South African *Cissampelos* species used for morphological and anatomical studies

Species	Voucher specimen(s) (all in UZ)	Locality
C. capensis (coastal form)	De Wet 2	Sedgefield [3422 BB (Mossel Bay)]
	De Wet 3	Still Bay [3421 AD (Riversdale)]
	De Wet 4	Mossel Bay [3422 AA (Mossel Bay)]
C. capensis (inland form)	De Wet 10	15km east of Ladismith [3321 AD (Ladismith)]
	De Wet 11	Clifton [3224 BC (Graaff-Reinet)]
C. hirta	De Wet 12	Mbibi [2632 DD (Bela Vista)]
	De Wet 17	Road to Lake Sibaya Lodge [2732 BC (Ubombo)]
	De Wet 19	Monzi, 15km east of Mtubatuba [2832 AC (Mtubatuba)]
C. mucronata	De Wet 33	15km east of Coastal Cashew Farm [2732 BC (Ubombo)]
	De Wet 34	Owen Sithole Agriculture College [2831 DB (Nkandla)]
C. torulosa	De Wet 41	University of Zululand campus [2831 DC (Nkandla)]
	De Wet 42	Owen Sithole Agriculture College [2831 DB (Nkandla)]
	De Wet 43	Gate to Sodwana, next to Lake Sibaya [2732 BC (Ubombo)]

Table 2: Morphological variation in the rhizomes and leaves of the four South African *Cissampelos* species

Character	*C. capensis* (inland form)	*C. capensis* (costal form)	*C. hirta*	*C. mucronata*	*C. torulosa*
Rhizome					
Size:	up to 35mm	up to 35mm	up to 20mm	up to 20mm	up to 15mm
Surface:	smooth	smooth	irregular	irregular	irregular
Shape:	long and cylindrical	long and cylindrical	long and cylindrical	long and cylindrical	irregular and abruptly tapering
Leaf lamina					
Shape:	ovate	ovate-deltoid	deltoid-cordate	ovate-cordate	reniform-deltoid
Apex:	rounded	obtuse-retuse	acuminate-retuse	acute-obtuse	obtuse-cordate
Base:	cuneate	cuneate-truncate	cordate-truncate	cordate-deeply cordate	cordate-truncate
Length:	up to 20mm	up to 40mm	up to100mm	up to 150mm	up to 75mm
Width:	up to 15mm	up to 35mm	up to 90mm	up to 145mm	up to 100mm
Texture:	coriaceous	coriaceous	coriaceous	velvety	papery
Surface hair:	glabrous to pilose	glabrous to pilose	glabrous to puberulent	puberulent to tomentose	glabrous to pilose
Colour (adaxial):	grey	bright green	dark green	pale green	dark green
Glossiness (adaxial):	not glossy	not glossy	glossy	not glossy	not glossy
Leaf venation:	3–5 nerved visible below	3–5 nerved visible below	5–7 nerved visible both sides	5–8 nerved visible both sides	3–5 nerved visible both sides
Petiole					
Length:	up to 10mm	up to 25mm	up to 45mm	up to 80mm	up to 85mm
Geniculate pulvinus:	absent	absent	present	absent	present
Tuft of hairs at petiole attachment:	absent	absent	absent	absent	present
Petiole attachment: position (distance from lamina base)	up to 1mm	up to 1mm	up to 2mm	up to 2mm	up to 1mm

strongly convex and sometimes appear papillate in *C. capensis*. Phellogen develops from the subepidermal layer of the primary cortex in older stems. The subepidermal collenchyma is three to nine layers thick with chloroplasts. Sclerenchyma fibres form arcs joined by stone cells in the vascular rays making a continuous ring. To the inside of the sclerenchyma, parenchyma caps are present, especially in young stems. Seven to ten vascular bundles are arranged in a circle. Broad primary medullary rays separate them.

Figure 2: Morphology of the rhizomes of the four South African species of *Cissampelos* (from left to right) *C. capensis* with smooth surface; *C. hirta* with irregular surface; *C. mucronata* with irregular surface; an exceptionally large specimen of *C. torulosa* with an irregular surface. Scale bar = 10mm

Large, conspicuous vessels characterise the xylem, with poorly defined phloem-like cells on the inside. Supplementary longitudinal sections, stained with 0.1% aniline blue and viewed with a florescence microscope, indicated that these were not phloem cells and they are therefore presumably protoxylem cells. The pith is composed of large parenchyma cells, which are lignified only in *C. capensis* (Figure 5A). Tanniniferous idioblasts are present in the cortex and pith of *C. hirta* and *C. mucronata* (Figures 5B, C).

The leaves are dorsiventral and vary in thickness from 0.1mm to 0.3mm. Bicellular hairs, similar to those described for stems, are present on both sides in *C. hirta* and *C. mucronata*, but only over the vascular bundles in *C. torulosa* and are absent in *C. capensis*. Stomata are restricted to the abaxial surface. The cuticle is very thin (<1mm) and only present on the adaxial side, except in *C. torulosa* where it is not apparent on either surface. The upper epidermal cells are larger than the lower ones, except sometimes in *C. torulosa*. The shape of the epidermal cells varies from round or periclinally elongated to irregular. The outer periclinal cell walls are flattened to slightly convex on the upper side and fairly strongly cutinised, except for *C. torulosa*, which is less cutinised. The inner periclinal and anticlinal cell walls are cutinised to a lesser extent except occasionally in *C. hirta* (Figure 6B). Papillae are found on the epidermal cells of both leaf surfaces in *C. capensis* (Figure 6A). Conspicuous amounts of mucilage are apparent (Figure 6) and may be either subepidermal or represent coalesced mucilaginous inner periclinal cell walls of the epidermal cells. This mucilage is present adaxially but rarely abaxially in *C.*

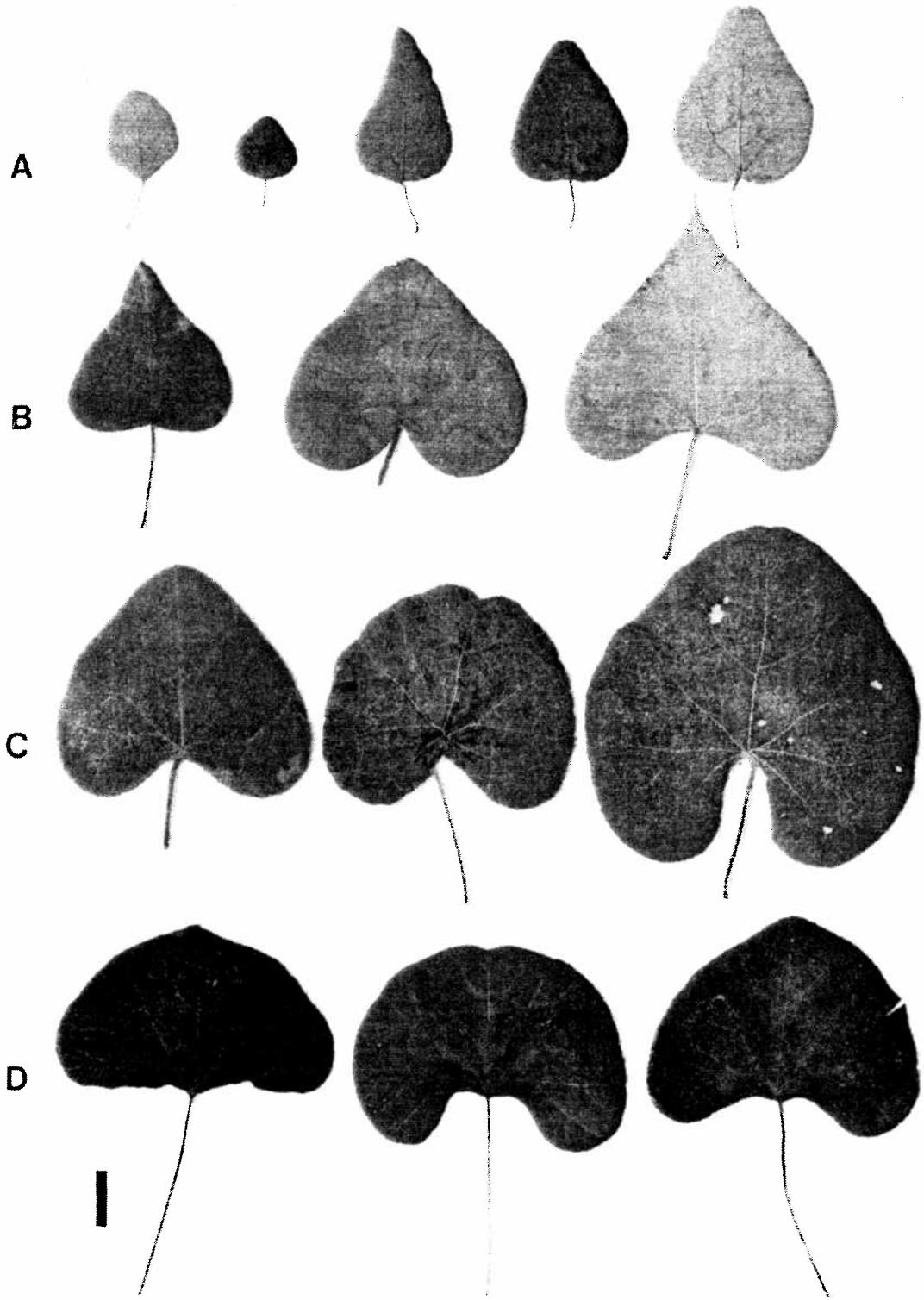

Figure 3: Morphological variation in the leaves of the four South African *Cissampelos* species (A) *C. capensis* – small, ovate (first two leaves, from inland habitats) or larger, deltoid (last three leaves, from coastal habitats); (B) *C. hirta* – deltoid to cordate with a coriaceous texture; (C) *C. mucronata* – ovate to cordate with a velvety texture; (D) *C. torulosa* – ovate to reniform, with very long petioles and a papery texture. Scale bar = 10 mm. Voucher specimens and localities (from left to right) as in Table 1 (A) De Wet 9, 11, 4, 5 and 1; (B) De Wet 23, 17 and 13; (C) De Wet 32, 33 and 34; (D) De Wet 41, 42 and 36

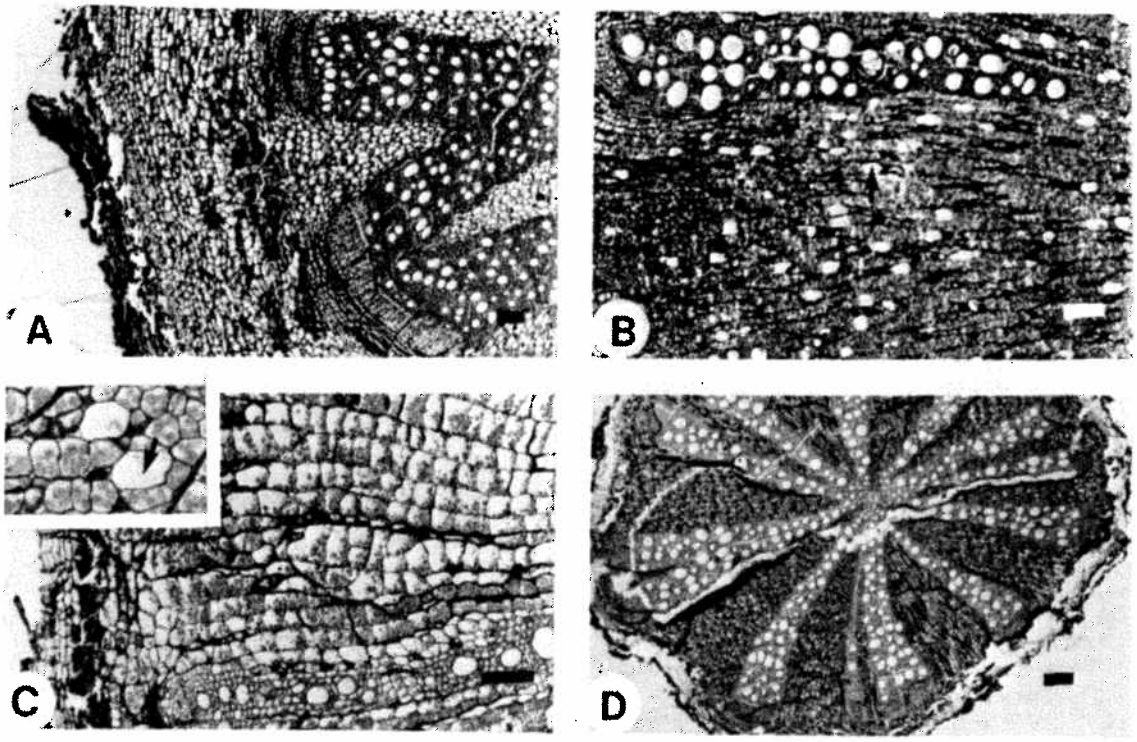

Figure 4: Light micrographs of the rhizomes of the four South African species of *Cissampelos* (TS), with broad medullary rays and large vessels (A) *C. capensis* (no secretory cells); (B) *C. hirta* (secretory cell indicated by arrow); (C) *C. mucronata* (insert: secretory cell indicated by arrow); (D) *C. torulosa* (no secretory cells). Scale bar = 120mm

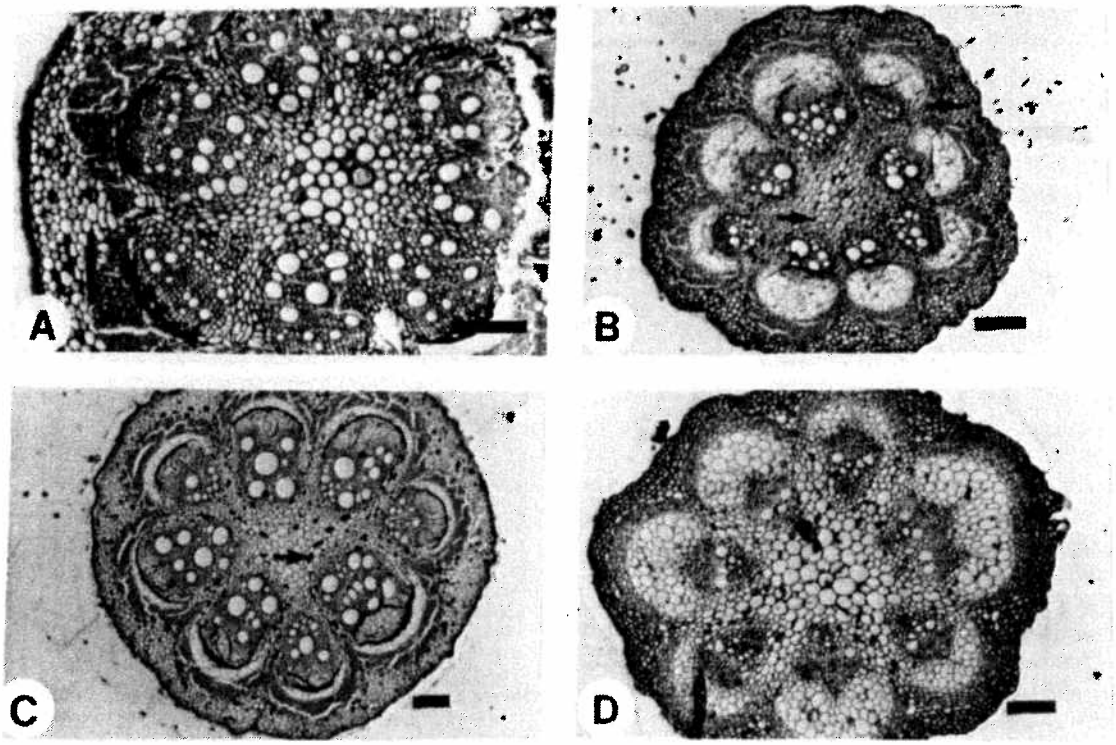

Figure 5: Light micrographs of the above-ground stem of *Cissampelos* (TS) (A) *C. capensis* with lignified parenchyma cells in the pith; (B) *C. hirta* (tanniniferous idioblast, indicated by arrow); (C) *C. mucronata* (tanniniferous idioblast, indicated by arrow), where sclerenchyma cap and stone cells form a continuous ring; (D) *C. torulosa* with a very thin cuticle. Scale bar = 120mm

capensis. Mesophyll is clearly differentiated into palisade and spongy parenchyma. The palisade usually consists of one cell layer only. Occasionally loosely packed cells of a second layer are found in plants growing in drier places being most developed in *C. capensis*. There is usually also a single, or occasionally a double, incomplete layer of palisade cells on the abaxial side in *C. capensis* (Figure 6A) so that the leaves are not as markedly dorsiventral as in the other three species. Palisade parenchyma cells are usually elongated and densely packed while the spongy parenchyma cells are more or less isodiametric and loosely packed. Characteristic, often slightly cutinised while interconnections between the spongy parenchyma cells are present. Elongated secretory cells, with unknown function, are generally associated with the vascular bundles as are tanniniferous cells in *C. hirta* and *C. mucronata*.

The midrib area is somewhat flattened above except in *C. torulosa* (Figure 7D) and occasionally in *C. mucronata* (Figure 7C) where it is slightly raised. It is conspicuously raised below in *C. mucronata* (Figure 7C) but less so in the other species (Figures 7A, B). Epidermal cell walls are highly cutinised especially in *C. capensis* with striae present on the adaxial side and occasionally on the abaxial side, but absent in *C. torulosa*. Epidermal cells are usually isodiametric and much smaller than those of the rest of the lamina except in *C. torulosa* (Figure 7D). The outer periclinal cell walls are strongly convex, resulting in the formation of papillae in *C. capensis* (Figure 7A). Subepidermal collenchyma is present. Sclerenchyma is usually found on the abaxial and adaxial sides of the vascular bundles, except in *C. capensis* where it is absent. Elongated tanniniferous idioblasts are scattered amongst the collenchyma cells of *C. hirta* and *C. mucronata* (Figures 7B, C). Vascular bundles are collateral.

The circumference is variable, from round or oval to reniform and sometimes with an undulating outline. Bicellular hairs (Figure 8C) similar to those described for stems are scattered on the epidermis, but rarely present in *C. capensis*. The cuticle is thin (<1mm) and the epidermal cells vary from round to square. Outer periclinal cell walls are usually flat to slightly convex but more convex in *C. capensis* giving a somewhat papillate appearance (Figure 8A). The outer walls are usually strongly cutinised and the inner periclinal and anticlinal cell walls are cutinised to a lesser extent. Stomata are present. The subepidermal collenchyma is three to eight cell layers thick. Sclerenchyma forms a continuous undulating ring outside the parenchyma surrounding the vascular tissue but it is absent in some *C. capensis* populations (De Wet 2 and 10, Table 1). Collateral vascular bundles are arranged in a circle and the number varies between five to seven, with those on the abaxial side being larger than the rest. Tanniniferous idioblasts are scattered throughout the petiole in *C. hirta* and *C. mucronata* (Figures 8B, C).

The anatomical study of the four *Cissampelos* species revealed definite differences between the species. These are summarised in Table 3.

Discussion

The data presented in Tables 2 and 3 are a summary of all features that are potentially useful to distinguish between plants and plant parts (rhizomes and leaves) of the four South African *Cissampelos* species and could serve as keys to the species. The diagnostic value of these characters is briefly discussed below.

It is the rhizomes of the four species that are mostly used for medicinal purposes. Morphologically *C. capensis* and *C. torulosa* rhizomes are easily identifiable — the former having a regular shape and smooth surface as opposed to an irregular shape and rough surface in the latter. Rhizomes of *C. hirta* and *C. mucronata* are irregularly fluted and are not distinguishable from one another. Although the rhizomes of *C. capensis* are much larger than those of C. *torulosa*, both have similar anatomical features, including the lack of secretory cells, which occur in *C. hirta* and *C. mucronata*.

Differences in leaf morphology enable *C. capensis* to be distinguished from *C. torulosa*, *C. hirta* and *C. mucronata* (see Tables 2 and 3). The latter two species are, however, very similar anatomically. Because of the variation in leaf shape within *C. hirta* and *C. mucronata* (Figure 3), it is difficult to use leaf shape and leaf base for distinguishing between them. The leaf apex can be useful as a diagnostic character; in *C. hirta* it is slightly acuminate and in *C. mucronata* it is slightly obtuse. In *C. hirta* the petiole is often distinctly grooved on the adaxial side, while it is usually only slightly grooved in *C. mucronata* (Figure 8). The only outstanding leaf features are the different textures and the presence of a geniculate pulvinus in *C. hirta* but not in *C. mucronata*. In mature fresh leaves of these two species, the colour difference is also clear. The leaves of *C. capensis* are much smaller than those of the other three species and easy to identify (Table 2 and Figure 3). The leaf size, shape and colour of the inland populations (where they grow in a much drier habitat), differ substantially from that of the coastal populations (Figure 3). The delicate dark green leaves of *C. torulosa*, with their reniform shape, distinguish it clearly from the other three species. A further difference between *C. hirta* and *C. mucronata* and the other two, is the presence of tanniniferous idioblasts in their laminas, petioles and stems. The shape of their midribs also differs from the other two species. The distinguishing characteristics of *C. capensis* are the absence of hairs on the leaves, the shape of the epidermal cells and the presence of mucilage on both abaxial and adaxial sides in the leaf, as well as the existence of a palisade layer on the abaxial side and lignified parenchyma cells in the pith. There seem to be no major anatomical differences between the coastal and interior populations of *C. capensis*, except for the more compact palisade cells in the interior populations. The rhizomes of the four species of this genus are easily recognisable as members of the family Menispermaceae. The rhizomes of *C. capensis* and *C. torulosa* can be readily identified but it is not possible to distinguish between those of *C. hirta* and *C. mucronata*.

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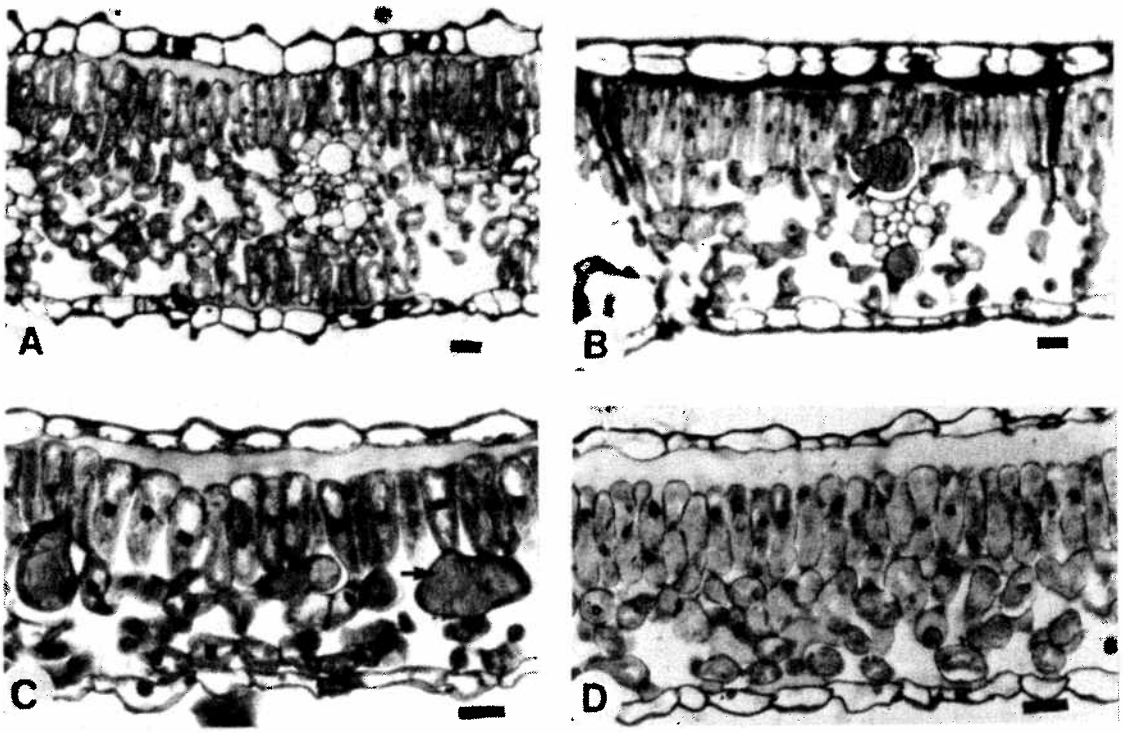

Figure 6: Light micrographs of the leaf lamina of *Cissampelos* (TS), with subepidermal mucilage in all four species visible (A) *C. capensis* with palisade layers on both adaxial and abaxial sides and papillae on the epidermal cells of both surfaces; (B) *C. hirta* (tanniniferous idioblast, indicated by arrow); (C) *C. mucronata* (tanniniferous idioblast, indicated by arrow); (D) *C. torulosa*. Scale bar = 20mm

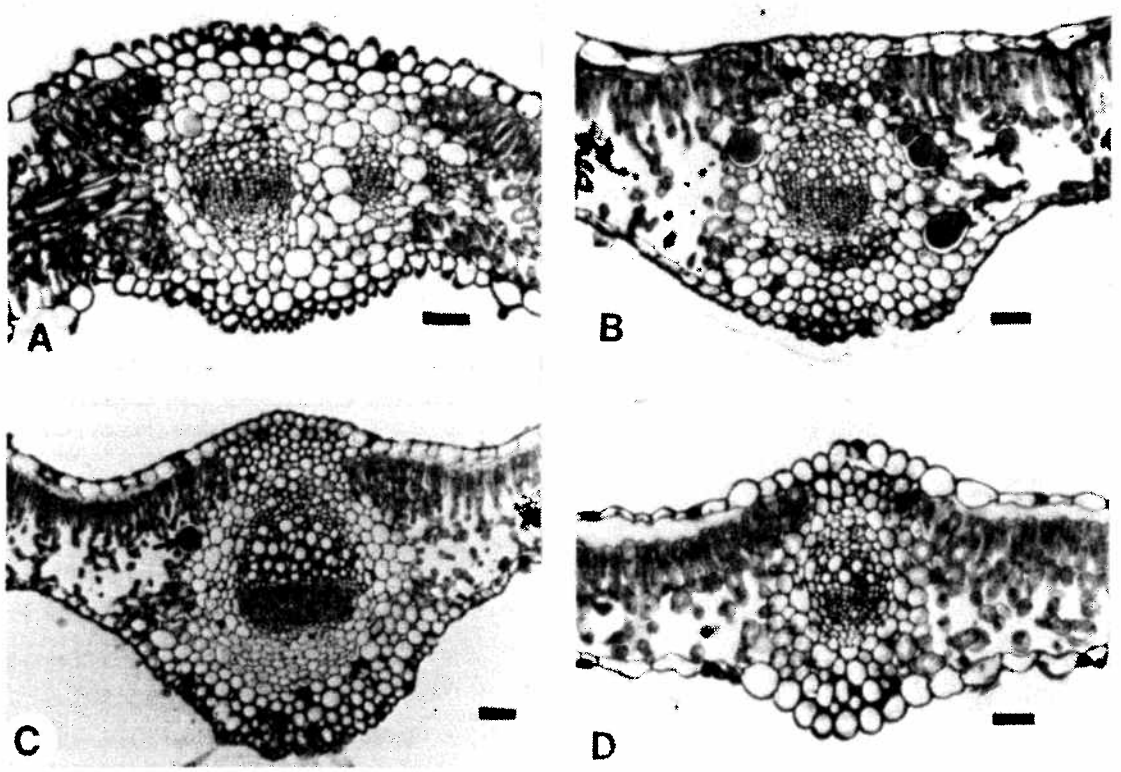

Figure 7: Light micrographs of the leaf midrib of *Cissampelos* (TS) (A) *C. capensis* with papillate surface; (B) *C. hirta* (tanniniferous idioblast, indicated by arrow); (C) *C. mucronata* (tanniniferous idioblast, indicated by arrow); (D) *C. torulosa* with large epidermal cells. Scale bar = 40mm

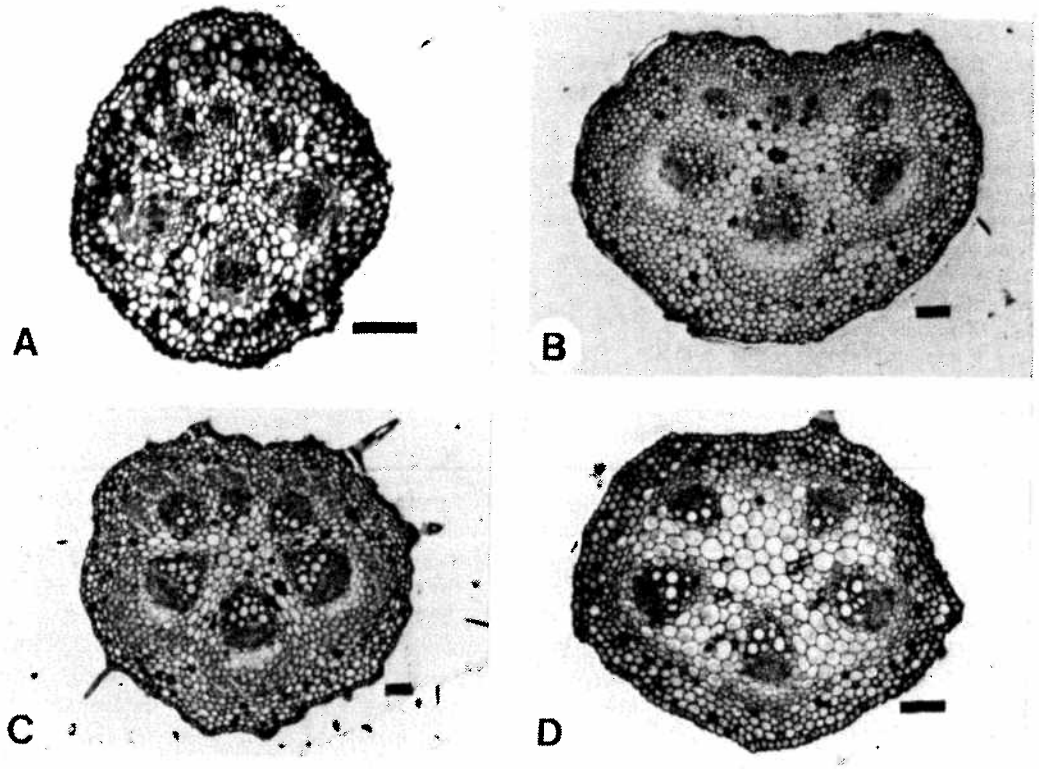

Figure 8: Light micrographs of the petiole of *Cissampelos* (TS), with collateral vascular bundles that vary in number from five to seven, with those on the abaxial side being larger in diameter than the rest (A) *C. capensis* with an oval outline; (B) *C. hirta* with a reniform outline; (C) *C. mucronata* with undulate outline showing bicellular hairs; (D) *C. torulosa* with an angular outline and flattened adaxially. Scale bar = 80mm

Table 3: Anatomical differences between the four South African *Cissampelos* species*

Character	*C. capensis*	*C. hirta*	*C. mucronata*	*C. torulosa*
Rhizome				
Secretory cells:	absent	present	present	absent
Above-ground stem				
Cuticle:	7mm	5mm	3mm	<2.5mm
Outer periclinal cell walls	present	absent	absent	absent
strongly convex appearing				
somewhat papillate:				
Parenchyma cells in pith lignified:	present	absent	absent	absent
Leaf				
Hairs:	absent	present	present	on veins only
Outer periclinal cell walls with	present	absent	absent	absent
papilla-like protuberances:				
Abaxial mucilage:	present	absent	absent	absent
Palisade cells on abaxial side:	present	absent	absent	absent
Midrib shape on adaxial side:	flattened	flattened	flattened	slightly raised
Midrib shape on abaxial side:	slightly raised	slightly raised	conspicuouslyraised	slightly raised
Presence of sclerenchyma cells	none	adaxial and abaxial	adaxial	adaxial and abaxial
on side of main vascular bundle:				
Elongated tanniniferous idioblasts	absent	present	present	absent
amongst collenchyma cells:				
Petiole				
Outline in TS:	round to oval	reniform	undulate	flattened dorsally
Outer periclinal cell walls strongly	present	absent	absent	absent
convex appearing somewhat papillate:				
Tanniniferous idioblasts in cortex and pith:	absent	present	present	absent

*No anatomical differences were observed between the coastal and inland forms of *C. capensis*

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