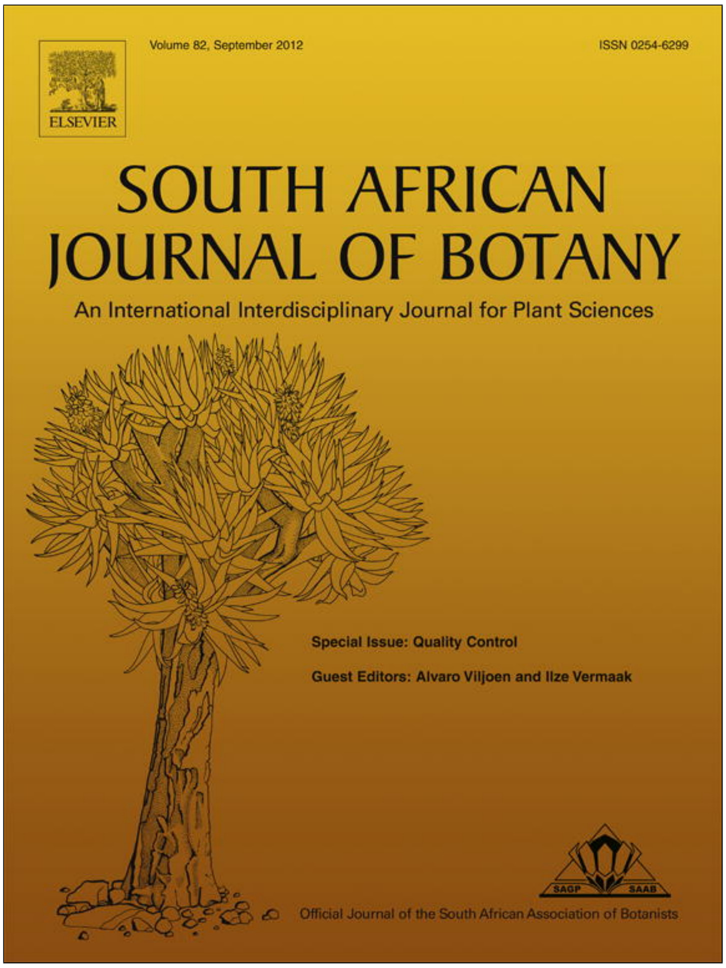
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Leaf and stem anatomy of honeybush tea (*Cyclopia* species, Fabaceae)

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Abstract

The leaf and stem microstructure of commercialised *Cyclopia* species (*Cyclopia intermedia*, *Cyclopia subternata*, *Cyclopia maculata*, *Cyclopia genistoides*) is described and illustrated for the first time. Leaflet characters of diagnostic value include the shape, margin (flat or revolute), bundle sheath extensions, crystals (aggregates, solitary crystals and crystal sand), relative size of the upper and lower epidermal cells and thickness of the cuticle. The study revealed an abundance of phenolic compounds, not only in the leaf epidermal cells and leaf bundle sheaths, but also in the secondary phloem, xylem rays, xylem parenchyma and pith, showing that the inclusion of older stems may be acceptable and does not necessarily lower the quality of the product.

Keywords: Crystals; *Cyclopia* species; Honeybush tea; Leaf anatomy; Pharmacognosy; Quality control; Stem anatomy

1. Introduction

The genus *Cyclopia* (Fabaceae, tribe Podalyrieae) is a group of 23 species of papilionoid legumes endemic to the fynbos region of South Africa (Schutte, 1997). It is the source of raw material for the commercial production of honeybush tea, a Cape herbal beverage that is currently making a come-back as a popular and tasty health drink (Joubert et al., 2011). The species are morphologically and chemically rather similar and most of them have been used from time to time in rural areas to produce tea. The plants are sprouting or non-sprouting woody shrubs with yellowish twigs, trifoliolate leaves, attractive yellow flowers (with the standard petal characteristically grooved) and brown pods bearing arillate seeds (Joubert et al., 2011; Schutte et al., 1995; Schutte, 1997; Van Wyk and Gericke, 2000; Van Wyk et al., 1997, 2009). The species all have mangiferin as major phenolic constituent (De Nysschen et al., 1996; Ferreira et al., 1998; Kamara et al., 2004), a xanthonoid of considerable medicinal interest, with gastro-protective (Carvalho et al., 2007) and numerous other pharmacological effects (see review of herbal teas by Joubert et al., 2008).

Cyclopia genistoides (L.) R.Br. (honeybush tea, Malmesbury to Albertinia area) has been used in the vicinity of Cape Town at least since the eighteenth century (Marloth, 1925; Pappe, 1847). *Cyclopia intermedia* E. Mey. (berg tea, Kouga berg tea, Witteberg to Tsitsikamma Mountains) and *Cyclopia subternata* Vogel (vlei tea, George and Tsitsikamma regions) gradually became the most important sources of tea but currently the commercial focus is again on *C. genistoides* (Joubert et al., 2011). Small quantities of tea have sporadically been made from *Cyclopia maculata* (Andrews) Kies (Genadendal tea, vlei tea, Bainskloof to Riversdale area), *Cyclopia meyeriana* Walp. (Cedarberg tea), and *Cyclopia sessiliflora* Eckl. & Zeyh. (Heidelberg tea, Warmwaterberg and Langeberg Mountains). The tea is traditionally made in the flowering period in spring, as the flowers are believed to add to the distinctive honey flavour and aroma.

The branches are wild-harvested in bundles and often have to be carried over long distances in mountainous terrain. Using a modified silage cutter, the distinctive yellowish stems and leaves are cut into small sections, after which they are bruised and moistened. The material is left to oxidize spontaneously, or is

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heated in an oven to about 60 °C to enhance the process. Enzymatic oxidation turns the chopped material into a rich brown colour and enhances the characteristic sweet, honey-like smell. After a few hours, the tea is spread out in the sun to dry (Joubert et al., 2011; Marloth, 1925; Van Wyk and Wink, 2004; Watt and Breyer-Brandwijk, 1962). Modern processing is done through rotary fermentation at controlled temperatures and drying artificially or in the sun (Joubert et al., 2011). Nowadays, unfermented (green) honeybush tea is also available.

The aim of this study was to explore the potential value of leaf and stem anatomical characters of *Cyclopia* species in pharmacognosy and quality control. We wanted to see if there are any anatomical characters that can be used to distinguish between commercial tea samples made from different species.

2. Materials and methods

Ten commercial tea samples and thirty-two authentic reference samples of stem and leaf material of *C. genistoides*, *C. intermedia*, *C. maculata* and *C. subternata* were used in this study. The provenances of the materials and voucher specimens are given in Table 1. The samples were collected at various

Table 1
Distribution of leaf anatomical characters of potential diagnostic value in 42 samples from four *Cyclopia* species. Voucher specimen abbreviations: ALS = A.L. Schutte; BvW = B.-E. Van Wyk; KK = E. Kotina.

Species, samples and provenances (voucher specimens in JRAU)	Crystals in aggregates	Solitary crystals	Crystal sand	Upper epidermal cells larger	Bundle sheath extension	Cuticle thick	Leaf margin revolute
C. genistoides:							
Commercial tea sample (tea bag)	+	+	+	+	−	+	+
J Vlok 2249 (Buffelshoek, Albertinia)	+	+	+	+	−	+	+
ALS 621 (Rooiels)	+	+	+	+	−	+	+
ALS 622 (Rooiels)	+	+	+	+	−	+	+
ALS 624 (Betty's Bay)	+	+	+	+	−	+	+
ALS 625 (Betty's Bay)	+	+	+	+	−	+	+
ALS 615 (Constantia Mountain)	−	−	+	+	−	+	+
BvW 2747 (Constantia Mountain)	−	+	+	+	−	+	+
C. intermedia:							
Commercial tea sample 1 (loose tea)	+	+	+	+	+	+	−
Commercial tea sample 2 (loose tea)	+	+	+	+	+	+	−
Commercial tea sample 3 (loose tea)	+	+	+	+	+	+	−
Commercial tea sample 4 (tea bag)	+	+	+	+	+	+	−
Commercial tea sample 5 (tea bag)	+	+	+	+	+	+	−
Commercial tea sample 6 (tea bag)	+	+	+	+	+	+	−
ALS 507a (Joubertina, Langkloof)	+	+	+	+	+	+	−
ALS 507b (Joubertina, Langkloof)	+	+	+	+	+	+	−
ALS 507c (Joubertina, Langkloof)	+	+	+	+	+	+	−
BvW 945 (K'Buku, Langkloof)	+	+	+	+	+	+	−
BvW 947 (K'Buku, Langkloof)	+	+	+	+	+	+	−
BvW 928 (Prince Alfred's Pass)	+	+	+	+	+	+	−
ALS 518a (Prince Alfred's Pass)	+	+	+	+	+	+	−
ALS 518b (Prince Alfred's Pass)	+	+	+	+	+	+	−
BVW 950 (De Vlug)	+	+	+	+	+	+	−
ALS 524a (Swartberg Pass)	−	+	+	+	+	+	−
ALS 524b (Swartberg Pass)	−	+	+	+	+	+	−
ALS 524c (Swartberg Pass)	−	+	+	+	+	+	−
Pienaar 6 (Swartberg Pass)	−	+	+	+	+	+	−
ALS 513 (Hoopsberg)	−	+	+	+	+	+	−
ALS 521 (Oudtshoorn district)	−	−	+	+	+	+	−
C. maculata:							
Commercial tea sample (loose tea)	−	−	−	+	+	+	+
ALS 528b (Riversdale)	−	−	−	+	+	+	+
BvW 895 (Riversdale)	−	+	+	+	+	+	+
C. subternata:							
ALS 503 (Witelsbos)	+	+	+	−	+	−	−
ALS 505 (Kareedouw Pass)	−	+	+	−	+	−	−
ALS 681 (Plettenberg Bay)	−	−	−	−	+	−	−
ALS 682 (Plettenberg Bay)	−	−	−	−	+	−	−
ALS 683 (Plettenberg Bay)	−	−	−	−	+	−	−
ALS 690a (George District)	−	+	+	−	+	−	−
ALS 690b (George District)	−	+	+	−	+	−	−

times of the year and included voucher specimens, bulk samples air-dried in paper bags and material preserved in formalin:acetic acid:alcohol (FAA). Commercial products and surfaces of leaves and shoots were studied in the dry condition, and also after rehydration in boiling water. Samples were studied under normal and polarised light. The identities of the commercial samples were verified by careful anatomical comparisons with authentic reference samples. Fragments of calyces are particularly useful. The commercial *C. maculata* sample from Genadendal was from the 2008 flowering season (harvested in the traditional way to include the flowers). This is no longer considered practical for commercial production due to poor yields. Anatomical sections were made from material preserved in formalin:acetic acid:alcohol (FAA) or from dried bulk samples that were soaked in FAA for a few days after rehydrating. The glycol methacrylate (GMA) method of Feder and O'Brien (1968) was used to prepare permanent slides of leaves, while both leaves and stems were sectioned using a freezing microtome (Leitz Lauda Kryomat 1700). Slides were made in duplicate: some stained with periodic acid-Schiffs reagent and toluidine blue, and some were left unstained. The shape and distribution of crystals were studied under polarised light.

Photographs and measurements were made using an Olympus ColorView Soft Imaging System and the Olympus Analysis Imaging Solutions (OASIS) programme.

3. Results

3.1. Appearance and composition of the commercial product

The honeybush tea samples consisted predominantly of fragments of wood, bark, stem tips and leaves (Fig. 1, A1-5). The single sample of *C. maculata* contained a large percentage of flowers (Fig. 1, A5), whereas the tea samples from other species included only minute amounts of generative organs (flower buds, flowers parts and pod fragments). The pieces of stems and leaves making up the bulk of the material of *C. genistoides*, *C. intermedia* and *C. subternata* are usually 3–5 mm long but up to 30 mm in the sample of *C. maculata*. The leaves are pale yellowish-brown to dark brown, with the upper surface markedly lustrous and unlike the dull lower surface. Stem portions in the fermented tea samples are dark brown and ribbed. Pieces of bark can easily be distinguished from leaf fragments by having white pieces of xylem adhering to the inner surface and ribs on the outer surface. Wood fragments are white but may become brown during processing. Similar to rooibos tea, the woody parts absorb phenolics extracted when water is added (Du Toit and Joubert, 1998). Similar quantities of leaf, stem and wood material were present but usually much smaller amounts of bark fragments. The single sample of *C. maculata* tea had (by mass) 74% flower parts, 15% leaf material and 11% stem fragments.

3.2. Stem structure

Young stems are ribbed in all the species (Fig. 1, B1, B2). The cuticle is thick. The epidermis may persist for a few years and consists of isodiametric to dome-shaped cells with yellow contents. The cortex is composed of four to six layers of thin-walled isodiametric cells, similar in size to the epidermal cells, and also with yellow contents. Solitary and aggregate prismatic and acicular crystals in sheaf-like aggregates are common in the epidermis and cortex of *C. intermedia* and *C. genistoides*, but are absent in the epidermis of *C. maculata*. No crystals were found in the epidermis or cortex of *C. subternata*. The crystals are likely to be composed of calcium oxalate, because they are dissolved during the staining process. Vascular bundles are collateral. Primary phloem fibres have thin to thick or very thick walls and commonly occur in groups of 10–20 (20–40 in *C. genistoides*). The pith may be round, oval or stellate, and is composed of parenchyma, commonly with yellow contents. Solitary and aggregate prismatic and acicular crystals in sheaf-like aggregates are usually present in some pith cells in all the species except *C. subternata*, where aggregate crystals were found in only one of the 11 samples studied (Table 1).

Older stems are grey and smooth. Initiation of the first-formed periderm is subepidermal (Fig. 1, B3). Subsequent periderms were not observed. The secondary phloem comprises sieve tubes, companion cells and phloem parenchyma. Sieve plates are simple. Axial parenchyma cells are fusiform and occur in strands of two to six cells. Rays are uniseriate or bi-seriate (rarely three-seriate). Uniseriate rays are composed mostly of upright cells while multiseriate rays have procumbent and square cells, and also upright cells forming one to five marginal rows.

The transition from non-collapsed to collapsed secondary phloem is conspicuous. Sclerification of the parenchyma cells starts in non-collapsed secondary phloem. In collapsed secondary phloem, sieve elements and companion cells are obliterated. Axial parenchyma cells occur as thin-walled chambered crystalliferous cells, fibre-like sclereids or strands of sclerified cells. Crystals occur inside sclereids as well. They are present as acicular needles in sheaf-like aggregates. Dilatation of secondary phloem is mostly radial (Fig. 1, B5). Dilated rays are extensively enlarged, mostly by tangential expansion and also by anticlinal divisions of ray cells resulting in rays of up to eight cells wide. Ray cells are sclerified, and often contain acicular crystals in sheaf-like aggregates.

Wood is diffuse-porous, with vessels in radial multiples close to the pith but becoming clustered or arranged in a dendritic pattern away from the pith as secondary growth progresses (Fig. 1, B3, B4). Fibres vary from thin- to very thick-walled. Rays are numerous and uniseriate or bi-seriate (Fig. 1, B4). Crystals and yellow contents are often present in the vessels and parenchyma cells.

3.3. Leaf structure

The leaflets are simple and sub-sessile. They may be obovate and flat (*C. intermedia* and *C. subternata*) or linear and incurved (Fig. 2), with revolute margins (*C. genistoides* and *C. maculata*). The cuticle is thick except in *C. subternata* (Fig. 2, B1-3). The epidermal cells have yellow contents and are similar in size in *C. subternata* but smaller abaxially than

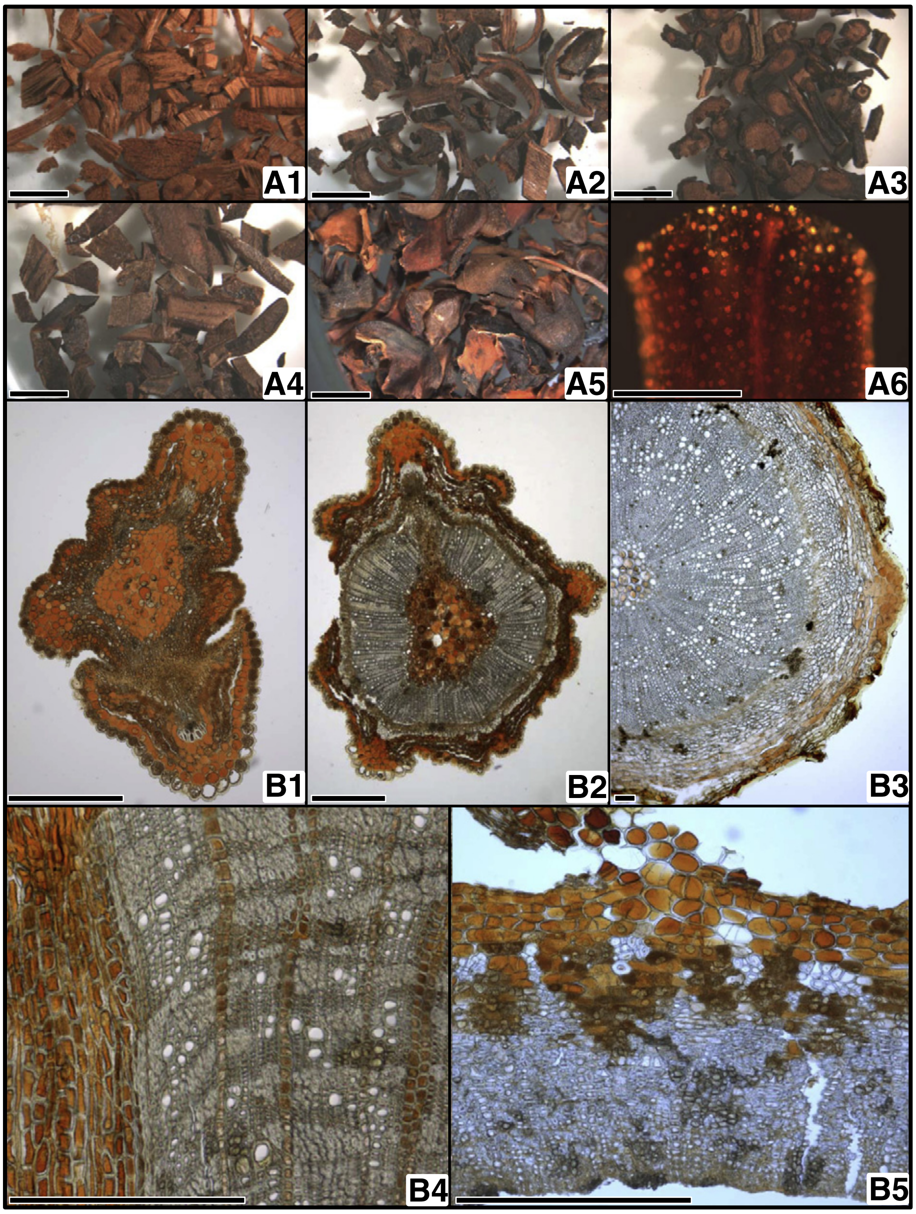

Fig. 1. The main components of honeybush tea (A1-6) and details of the stem anatomy (B1-5). Tea components of *Cyclopia intermedia* (A1-6): A1, wood; A2, bark fragments; A3, stem sections, A4, leaflet fragments. Tea components of *C. maculata*: A5, flower parts. A6, leaf fragment of *C. genistoides* under polarised light (note the abundance of crystals). Stem anatomy of *C. genistoides* (B1-5): B1, young stem showing abundance of phenolic compounds; B2, older stem showing phenolic compounds in the cortex and pith; B3, old stem showing dendritic arrangements of vessels and phenolic compounds; B4, xylem of old stem (note phenolic compounds in ray cells); B5, dilated cortex and secondary phloem. A1-5 from commercial samples; A6, B1-5 from *Schutte 624*; Scale bars: A1-5 = 5 mm; A6, B1-5 = 0.5 mm.

adaxially in the other species (Fig. 2, A3–D3). Stomata are confined to the abaxial side and are sunken (Fig. 2, C1, D1). The mesophyll is composed only of palisade parenchyma. The bundle sheath cells are large and parenchymatous with yellow contents. A bundle sheath extension is present on both sides of the main vein of all species except *C. genistoides*. Acicular crystals in sheaf-like aggregates are found in the epidermis and mesophyll in *C. intermedia* and *C. genistoides* (Fig. 1, A6) as well as in the bundle sheath cells of *C. genistoides* (Fig. 2, A2, A3, C2, C3). Crystals are usually absent in *C. subternata* and *C. maculata* (Fig. 2, B2, D2).

4. Discussion and conclusions

The leaves of the four commercial *Cyclopia* species studied are markedly dissimilar in several characters, so that leaf fragments can easily be used to identify them, provided that a light microscope is available. This is particularly useful

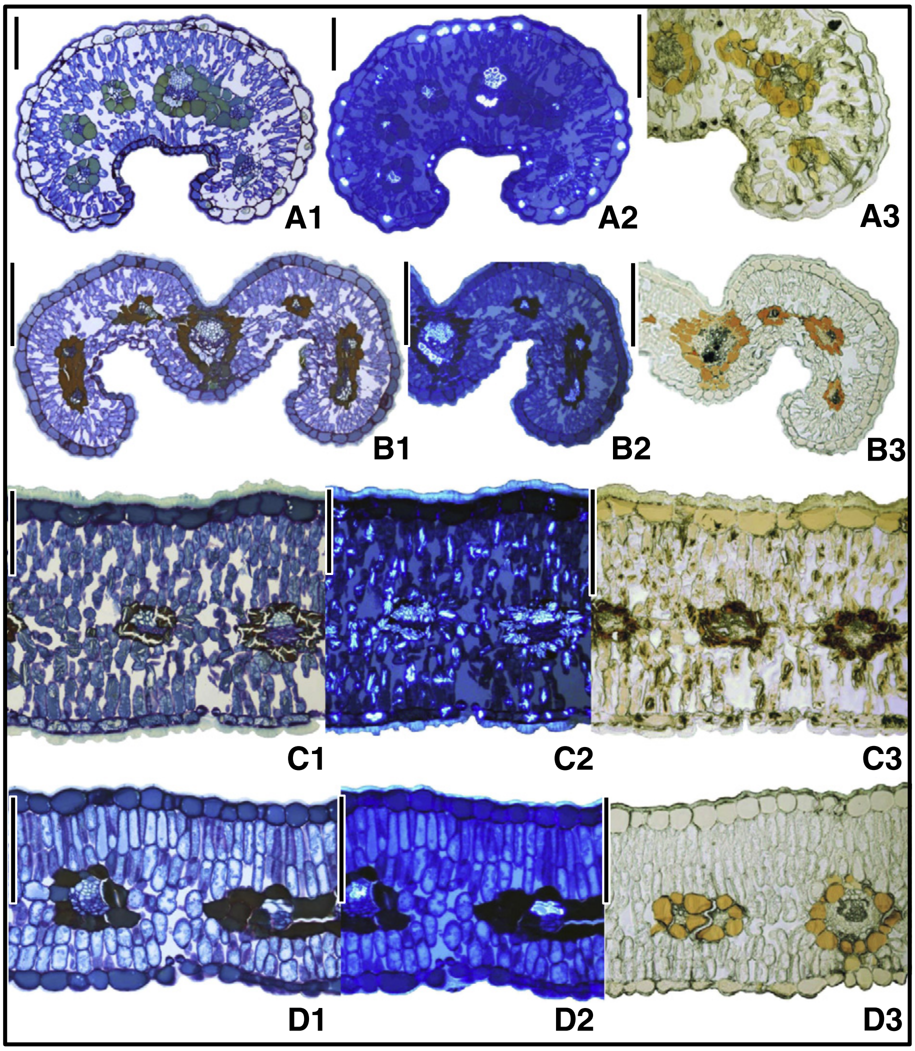

Fig. 2. Details of the leaf anatomy of *Cyclopia genistoides* (A1-3), *C. maculata* (B1-3), *C. intermedia* (C1-3) and *C. subternata* (D1-3). 1, stained with toluidine blue; 2, unstained, observed under polarised light; 3, unstained, observed under normal light. Note the abundance of crystals in *C. genistoides* (A2) and *C. intermedia* (C2) and their absence in *C. maculata* (B2) and *C. subternata* (D2). Other diagnostic characters include the thickness of the cuticle (thin in *C. subternata*), the relative size of the upper and lower epidermal cells (similar in *C. subternata*). A1-3 from *Schutte 624*; B1-3 from *Schutte 636*; C1-3 from *Schutte 678*; D1-3 from *Kotina 43–11*. Scale bars: A1–B3 = 0.5 mm; C1–D3 = 0.2 mm.

to distinguish between *C. genistoides, C. intermedia* and *C. subternata*, the three main commercial species. However, several other species are being developed as commercial sources of tea, so that a wider study will become necessary. Crystals are best observed on the upper leaflet surfaces because the stomata also tend to show up in polarised light:

1a. Margins of leaflets strongly revolute; leaflets linear-acicular, narrow (1–2 mm wide):

2a. Leaflet lamina not longitudinally grooved above; bundle sheath extension absent; crystals almost invariably present ……………………………………………………*C. genistoides*

2b. Leaflet lamina longitudinally grooved above; bundle sheath extension present; crystals usually absent ……………………………………………………………… *C. maculata*

1b. Margins of leaflets slightly revolute; leaflets oblong-ovate, broad (>2 mm wide):

3a. Upper epidermal cells markedly larger than lower ones; cuticle thick; crystals almost invariably present ……………………………………………………*C. intermedia*

3b. Upper epidermal cells similar in size to lower ones; cuticle thin; crystals usually absent ……………………*C. subternata*

Previous anatomical studies (Greenish, 1881; Metcalfe and Chalk, 1950) described some aspects of the leaf anatomy of *C. genistoides* but the details given are not sufficient to allow for comparative studies or identification at species level.

Yellow or orange cell contents, indicating the presence of phenolic compounds, are present in the epidermal and mesophyll cells of the leaves and are especially prominent in the bundle sheath parenchyma cells. Particularly noteworthy is the occurrence of yellow or orange inclusions in practically all parenchyma cells in stems, including the epidermis, primary phloem, secondary phloem, rays and pith. This means that even thick stems can be used as tea, because they would contribute substantially to the strength of the brew. There may be chemical differences between young and old stems and the quality of tea of older stems may be inferior to that made from leaves and/or younger stems. Modern consumers prepare honeybush tea as an infusion (tea) rather than as a traditional decoction (boiled for a lengthy period), so that a large percentage of stems may be considered undesirable because of the slower infusion rate. In contrast, the reddish brown cell contents in rooibos tea (*Aspalathus linearis*) are confined to the epidermal cells of the leaves and young stems only. Thicker rooibos tea stems therefore serve little more than to bulk up the volume of the product although some phenolics are absorbed into the outer layers of the wood during processing.

This appears to be a first attempt at describing the structure of honeybush tea (the commercial product) in terms of stem and leaf anatomy. The study has yielded some interesting new insights into diagnostic characters and may also have an impact on harvesting and production methods, because the phenolic substances occur in abundance in older stems.

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