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Anatomy of the leaf and bark of *Warburgia salutaris* (Canellaceae), an important medicinal plant from South Africa

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ABSTRACT

Bark and leaves of *Warburgia salutaris* are commonly used in traditional and modern herbal medicine but there are no published anatomical descriptions that can be used in pharmacognosy or in comparative anatomy. Descriptions of salient features are presented, showing that a combination of anatomical characters is of diagnostic value. Leaf material can be identified by the absence of trichomes and the presence of translucent secretory cells, thick adaxial cuticles, numerous small druse crystals in the epidermal cells, scattered large druses and mesophyll cells with brown contents. Bark is similarly characterized by the combination of secretory cells, druses, parenchyma cells with brown contents, thin-walled fibre-like sclereids and compound sieve plates located on the lateral walls and oblique cross walls of the sieve tubes.

1. Introduction

Warburgia salutaris (G. Bertol.) Chiov. (Canellaceae) is a popular medicinal plant distributed in eastern and southern Africa. The plant is an evergreen tree with fissured bark, simple, glossy leaves, green flowers and plum-shaped fruits (Codd, 1976; Coates Palgrave, 2002). The bark and leaves possess antimicrobial activity (Rabe and Van Staden, 1997, 2000; Mohanlall and Odhav, 2009; Kuglerova et al., 2011) and are commercially available in a crude form (usually bark) from local muti markets and as over-the-counter products (containing leaves or leaf extracts) from pharmacies. *Warburgia* is widely used in traditional medicine to treat coughs, colds, bronchial infections, oral thrush, cystitis and many other ailments (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1993; Hutchings et al., 1996; Neuwinger, 2000; Van Wyk et al., 2009). Fig. 1 shows bark samples on a traditional market (Fig. 1A) and examples of commercial bark and leaf samples (Fig. 1B–E).

The chemical composition of the bark and leaves is relatively well-studied. The bark is rich in essential oil and contains numerous drimane sesquiterpenes, including warburganal, mukaadial, salutariolide, polygodial and isopolygodial (Mashimbye et al., 1999; Drewes et al., 2001), as well as muzigadial (Rabe and Van Staden, 2000). A comparison of bark and leaves has shown that they are chemically similar in their terpenoid composition and that, in the interest of conservation, leaves can be sustainably produced as a replacement for bark (Zschocke et al., 2000; Drewes et al., 2001).

Despite its importance in traditional medicine, the bark of *W. salutaris* is anatomically poorly known (Metcalfe and Chalk, 1950) although the microstructure of young stems of *Warburgia stuhlmannii* Engl. and *Warburgia ugandensis* Sprague has been described by Wilson (1965). Thick bark from mature trees is preferred by herbalists as it is considered to be more potent and effective (Cunningham, 2001; Williams et al., 2007). The tree has been heavily exploited for its bark and there are concerns about the conservation of the species (Botha et al., 2004). In recent years, commercial products have become available from cultivated sources, especially bark harvested from thin branches (Fig. 1C) and also leaves (which are used as replacement for bark) (Fig. 1D, E).

Although anatomical descriptions are available for some species of *Warburgia*, the anatomy of the bark and leaves of *W. salutaris* have not yet been described. With recent emphasis on quality control and the safety of herbal preparations, such basic information may be of value in pharmacognosy. The aim of this study was to accurately describe the anatomy of bark and leaf samples and to identify characters of potential diagnostic value, not only to facilitate future comparisons with other species of *Warburgia* but also to allow for comparisons of anatomical characters within the Canellaceae.

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Fig. 1. Commercial products of *Warburgia salutaris*. A and B, bark as it is sold on traditional markets (arrows show *Warburgia* bark amongst several other bark samples), with B showing outer (left) and inner bark; C, commercial bark harvested from young cultivated trees; D, leaf powder; E, dried leaves.

2. Materials and methods

We studied fresh and dried bark and leaves of *W. salutaris*. Fresh material was collected in a private garden. Dried mature bark samples were purchased from the Faraday Muti Market in Johannesburg, while dried young bark and leaves were obtained from cultivated trees of a commercial grower.

Sections of bark were prepared from fresh material (temporarily stored in water, with no fixing fluid). Three pieces of stem (20–100 mm long) were cut from (1) branch tips without a visible periderm layer; (2) lower parts of the stem where periderm was starting to form; (3) thicker stems with mature bark that had more or less thick periderm. Transverse, radial, and tangential sections (20–40 μm thick) of stems were cut with a freezing microtome (Ernst Leitz GMBH, Wetzlar, Germany). Transverse sections were made with a freeze-microtome. Some sections were stained with a 1:1 alcian blue/safranin mixture (Jansen et al., 2004) and others were left unstained. Stained and unstained sections were examined and mounted in Euparal or glycerol. Maceration of secondary phloem was carried out in Jeffrey's solution for 24 h (Johansen, 1940). Lengths and diameters of sieve-tube members were determined from the macerated material mounted in glycerol. In the case of leaves, the material was placed in FAA for about 24 h and then treated according to the method of Feder and O'Brien (1968) for embedding in glycol methacrylate (GMA). Transverse sections, about 3–5 μm thick, were cut using a Porter–Blüm ultramicrotome. Sections were stained using the periodic acid-Schiff/toluidine blue method (Feder and O'Brien, 1968). Dry materials were studied before and after softening in hot water, followed by the same procedures as used for fresh material. Sections were photographed with an Olympus ColorView Soft Imaging System and measurements were taken with the Olympus Analysis Imaging Solutions (OASIS) programme. The descriptive terminology for bark structure follows Trockenbrodt (1990) and Junikka (1994).

3. Results

3.1. Leaf structure

Trichomes are absent. Epidermal cells have a polygonal shape in surface view (Fig. 2H). Anomocytic stomata are frequent on the abaxial surface and are diffusely arranged (Fig. 2H). When leaves are viewed in transmitted light, translucent spots, indicating secretory cells in the mesophyll (Fig. 2B,D), can be seen especially on the abaxial side. Small crystals are visible under polarised light (Fig. 2G).

The cuticle on the adaxial surface is thick (mean 5.0 μm) and thinner on the abaxial side (mean 2.4 μm). The epidermal cells are isodiametric to oval in shape (in transverse section) and contain a single small druse crystal (1.9–6.2 μm in diameter) (Fig. 2C, E). The mesophyll is distinctly differentiated into palisade and spongy parenchyma (Fig. 2E). Some of these cells have brown contents (presumably tannins). Druse crystals, larger than in the epidermis (10–21 μm in diameter), occur as idioblasts in the mesophyll (Fig. 2F). Large, globose secretory cells are also found in the mesophyll, particularly in the spongy parenchyma (Fig. 2E).

3.2. Bark structure

The surface of young stems is smooth, glabrous and green. The epidermis on young parts of stems consists of a single layer of isodiametric

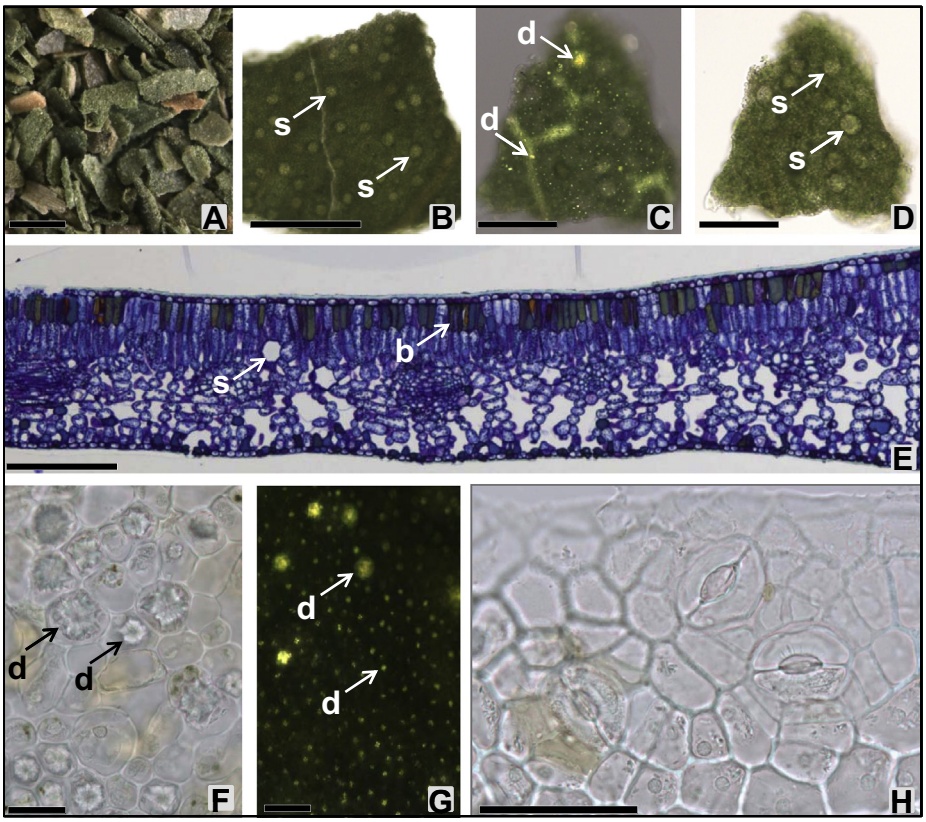

Fig. 2. Leaf anatomical characters of *Warburgia salutaris*. A, leaf powder; B, fragment from leaf powder showing secretory cells; C, leaf fragment under polarised light showing numerous small and a few large druse crystals; D, same leaf fragment under normal light; E, leaf in transverse section (note the thick cuticle); F, druse crystals in palisade parenchyma cells; G, leaf under polarised light showing numerous small and a few large druses; H, abaxial leaf surface showing stomata and epidermal cells with polygonal shape. Arrows: secretory cells = s; druses = d; cells with brown contents = b; Scale bars: A = 1 mm; B–G = 0.2 mm; H = 0.05 mm.

to oval cells. The cortex is composed of 10 to 20 layers of isodiametric to somewhat radially-flattened, thin-walled parenchyma cells. Two types of parenchyma cells can be distinguished, viz. small cells 10 to 40 μm in tangential diameter, and larger cells (idioblasts) 40 to 60 μm in tangential diameter (Fig. 3A). Some idioblasts are surrounded by axial parenchyma sheaths of one cell layer. The smaller parenchyma cells may contain starch grains, chloroplasts and/or brown contents (presumably tannins). Dilatation of the cortical tissue is effected mostly by tangential stretching of cells and also by the anticlinal division of the cortical parenchyma cells, thus forming strands of two to six cells. The cortical cells remain unsclerified and many contain druses, the number of which increases with age. Primary phloem fibres occur as solitary cells or in tangentially stretched groups of two to six cells and have slightly sclerified thin walls.

Mature bark is brittle with a shallow-fissured surface (Fig. 1B). The initiation of first-formed periderm is subepidermal. The phellem is composed of five to 10 layers of radially-flattened thin-walled cells with brown contents. The phelloderm comprises two to eight layers of radially-flattened cells with thin or U-shaped thickened walls, light brown contents and starch grains. Subsequent periderms are initiated in the outer region of the cortical parenchyma and form scales which comprise the rhytidome (Fig. 3D). The phellem is composed of five to 30 layers of radially-flattened, thin-walled cells, sometimes with light brown contents. The phelloderm comprises three to 10 layers of radially-flattened cells with U-shaped thickened walls and dark brown contents. The scales between periderm layers are large and can be >20 mm wide and >3 mm high. They are composed of dilated cortex or secondary phloem with rich brown contents. The secondary phloem is composed of tangential zones comprising sieve elements and companion cells which alternate with axial parenchyma cells, and are permeated by a network of phloem rays (Fig. 3B). Dilatation of secondary phloem is usually diffuse-radial or sometimes radial. Sieve tube members are 15–26 μm wide and 225–780 μm long. Sieve plates are compound and located on oblique cross walls (with eight to 30 perforations) (Fig. 3E, F). Sieve plates occur on the lateral walls (sometimes along the entire length of the cell) (Fig. 3F). Axial parenchyma cells are fusiform and in strands of two to 15 cells (rarely up to 20). Some strands contain one (rarely two) large secretory cells (idioblasts) (Fig. 3F).

Secondary phloem rays may be uniseriate or multiseriate (two to four cells thick) (Fig. 3F). Uniseriate rays are composed mostly of square cells while multiseriate rays have procumbent cells and sometimes square cells forming one marginal row. Ray parenchyma cells have brown contents and starch grains, and some cells contain druse crystals. Secretory cells (idioblasts) are present in the multiseriate rays. The transition from non-collapsed to collapsed secondary phloem is gradual. Collapsed secondary phloem is characterized by alternating zones of thin-walled fibre-like sclereids and axial parenchyma cells with brown contents. It is permeated by phloem rays with brown contents. Dilated rays are extensively enlarged, mostly by tangential expansion and also by anticlinal divisions of ray cells, resulting in rays of up to 15 cells wide. The number of druses increases with age.

Characters of potential value in identifying raw material of *W. salutaris* are summarized in Table 1.

4. Discussion

The bark anatomy of *W. salutaris* is similar to that of other members of the Canellaceae examined by Wilson (1965), but cells with brown contents and secretory cells in the secondary phloem were not described. *W. stuhlmannii* and *W. ugandensis* were recorded as having three to five phelloderm cell layers whereas *W. salutaris* appears to

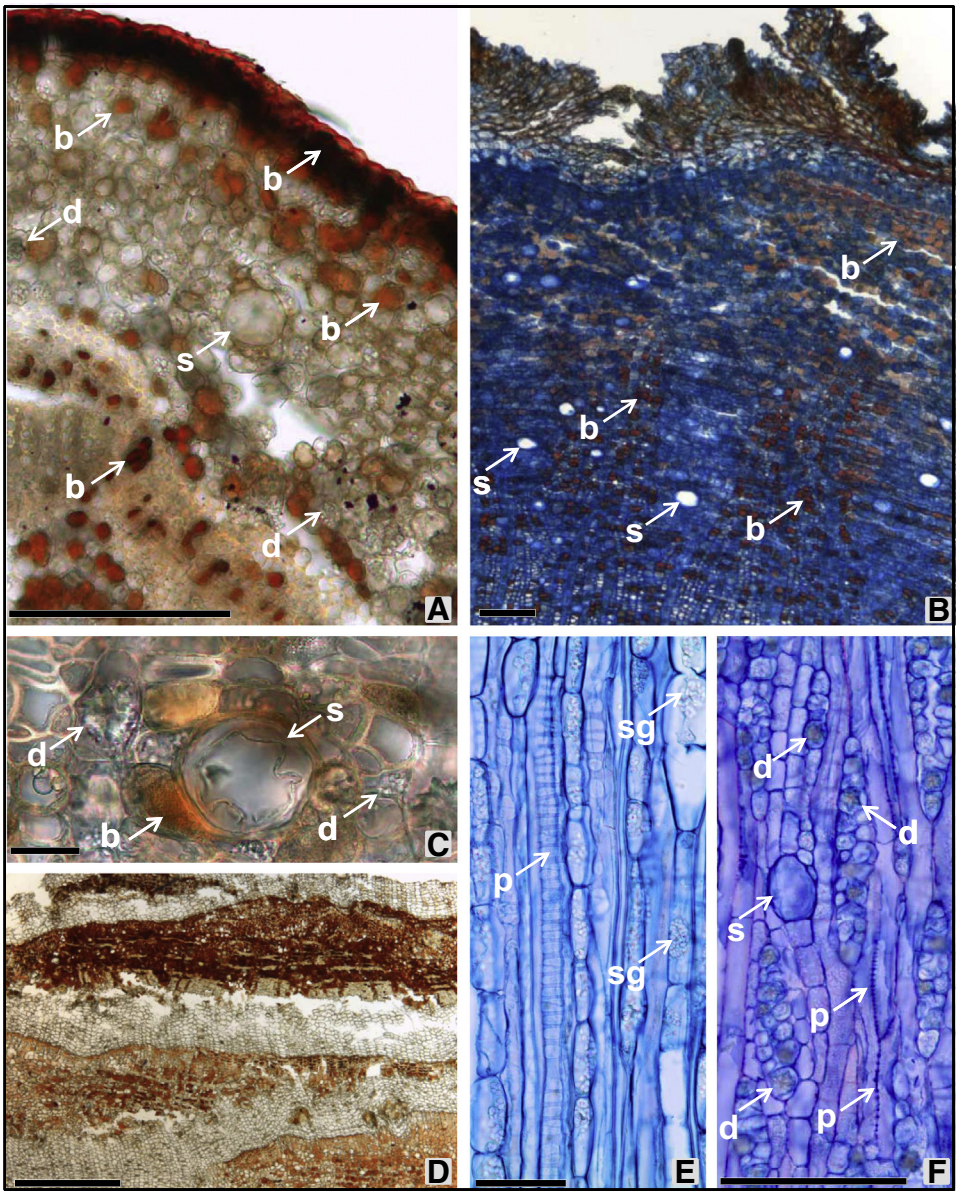

Fig. 3. Bark anatomical characters of *Warburgia salutaris*. A, young bark; B, mature bark; C, secretory cell in secondary phloem; D, rhytidome showing layers of periderm (phellem and phelloderm) with collapsed secondary phloem; E, radial section of secondary phloem showing compound sieve plates and starch grains in axial parenchyma cells; F, tangential section of secondary phloem showing secretory cell, axial parenchyma cells with druse crystals and sieve tubes with perforation plates also on the lateral walls. Arrows: secretory cells = s; druses = d; cells with brown contents = b; perforation plates = p; starch grains = sg. Scale bars: A and D = 1 mm; B, E, and F = 0.2 mm; C = 0.02 mm.

have two to 10 layers. In mature, thick bark, numerous parenchyma cells are present with brown contents. Particularly noteworthy are the prominent oil-containing idioblasts (secretory cells). The exact location of various secondary metabolites reported from *Warburgia* bark needs further study but it seems likely that the monoterpenes and various drimane sesquiterpines and others are present in the secretory cells. Similarly, the brown contents of the parenchyma cells are likely to be tannins or polyphenols. Secretory cells and brown (tannin-containing?)

Table 1
Morphological and anatomical characters of importance to identify leaf and bark material of *Warburgia salutaris*.

Diagnostic characters	Location
Trichomes	Absent from leaves and young stems
Thick cuticle	Leaf surface
Polygonal shape of epidermal cells	Leaf surface
Oil cells (idioblasts)	Stem cortex, secondary phloem (axial parenchyma, rays); leaf mesophyll
Large druse crystals (10–21 μm in diameter)	Stem cortex, secondary phloem rays and leaf mesophyll
Small druse crystals (1.9–6.2 μm in diameter)	Leaf epidermis
Parenchyma cells with brown contents	Stem cortex, secondary phloem (axial parenchyma, rays); leaf mesophyll
Thin-walled fibre-like sclereids	Secondary phloem
Sieve areas	On lateral walls and oblique cross walls of sieve tubes in secondary phloem
Subsequent periderms (rhytidome)	Mature bark

parenchyma cells are found in the leaves as well, so that the substitution of bark with leaves seems justified. Drewes et al. (2001) showed that the two biologically active ingredients, warburganal and poligodial, are present in both the leaves and the bark. It is noteworthy that leaves are used in traditional medicine in East Africa (Kokwaro, 1993). However, it is possible that the bark contains other compounds (or different combinations of compounds — see Kuglerova et al., 2011) that are collectively responsible for some of the claimed health benefits.

5. Conclusions

Several noteworthy anatomical features are present in the bark and leaves of *W. salutaris*, of which the combination of secretory cells, druse crystals of different sizes and parenchyma cells with brown contents seems particularly valuable for diagnostic purposes. These characters can be used to detect possible adulterants and contaminants in commercial samples. Leaves are distinct in having translucent secretory cells, no trichomes, epidermal cells with thick cuticles and single small druse crystals, as well as mesophyll cells with brown contents. Bark of young shoots can be recognized by the absence of trichomes, the presence of secretory cells, druse crystals, parenchyma cells with brown contents and occasional presence of thin-walled primary phloem fibres. Mature bark can be identified by the distinctly thick brown rhytidome with druse crystals, secretory cells in secondary phloem, parenchyma cells with brown contents, thin-walled fibre-like sclereids and compound sieve plates (with eight to 30 perforations) located on oblique cross walls and on the lateral walls of the sieve tubes.

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