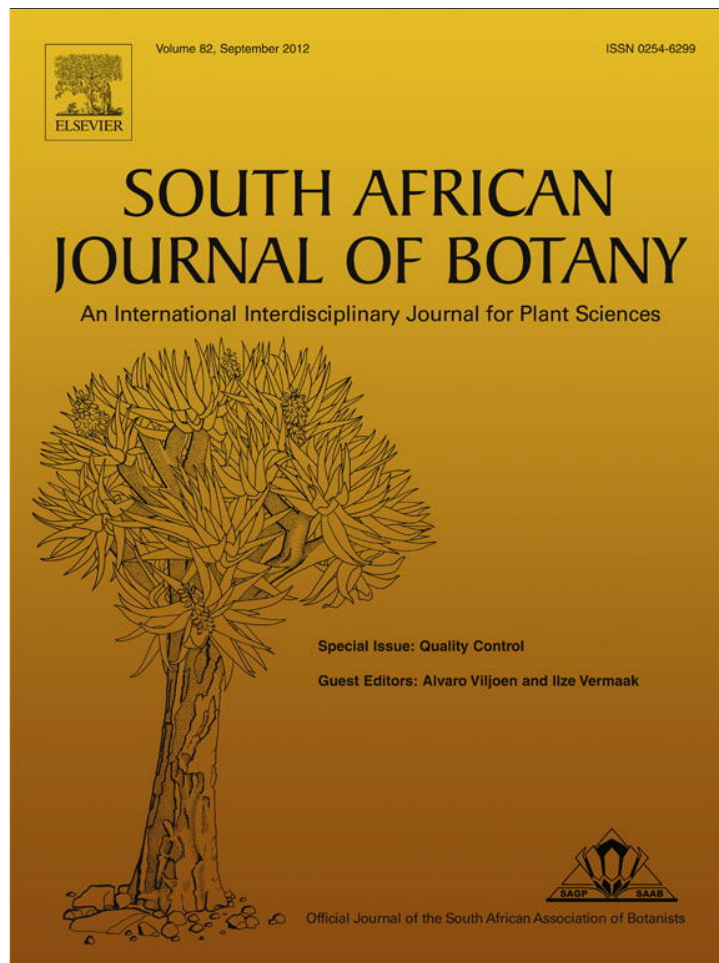


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Short communication

The pharmacognostic value of leaf and stem anatomy in rooibos tea (*Aspalathus linearis*)

E.L. Kotina^{a,b}, A.V. Stepanova^{a,b}, P.M. Tilney^a, B.-E. Van Wyk^{a,*}^a Department of Botany and Plant Biotechnology, University of Johannesburg, P.O. Box 524, Auckland Park 2006, Johannesburg, South Africa^b Komarov Botanical Institute of the Russian Academy of Science, Prof. Popov Str. 2, 197376 St. Petersburg, Russia

Available online 3 August 2012

Abstract

Aspalathus linearis (Fabaceae) is an exceptionally polymorphic species comprising seven distinct infraspecific forms, some reseeding and some resprouting after fire. We present, for the first time, anatomical data to describe the stems and leaves that are used to produce the well-known herbal product, rooibos tea. Comparisons between the commercial (cultivated) red tea type and selected wild types showed limited anatomical variation within the species. The cut and fermented leaves of the commercial form and various wild forms of the species show considerable variation, ranging from reddish brown to various shades of brown, grey and black. Infusions or decoctions are less variable and are bright reddish brown. Characters that may be useful for pharmacognostic purposes are the terete leaf segments, the similarity between leaf and stem sections, the thick cuticle, the bright yellowish brown colour of the epidermal cells (of leaves and young stems), the anomocytic stomata, the ring of small vascular bundles in the leaf and the highly sclerified main vascular bundle of the leaf.

© 2012 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Anatomy; *Aspalathus linearis*; Leaf anatomy; Pharmacognosy; Rooibos tea; Stem anatomy

1. Introduction

Rooibos tea is made from the cut stems and leaves of *Aspalathus linearis* (Burm.f.) R. Dahlgren (Fabaceae, tribe Crotonaceae), a woody shrub endemic to the western parts of the Cape region in South Africa, extending from Cape Town northwards to Nieuwoudtville (Dahlgren, 1968, 1988). The species is extremely variable. Based on growth habit, fire-survival strategy, leaf colour, flower colour and phenolic compounds, seven main biotypes were recognised (Van Heerden et al., 2003) as shown in Table 1. The two main fire-survival strategies in fynbos legumes have been discussed by Schutte et al. (1995). Plants from non-sprouting populations are killed by fire and regenerate from seeds only, while plants from sprouting populations coppice from their woody basal parts after fire (only the above-ground parts are killed). Studies of genetic (Van der Bank et al., 1995, 1999) and ecological

(Hawkins et al., 2011) variation in the species complex showed that sprouting and non-sprouting wild tea types are distinct entities. The main commercial type is the so-called red type or Rocklands type, a densely branched non-sprouting form with uniformly yellow flowers and bright green leaves that turn a rich red-brown colour after “fermentation” (actually enzymatic oxidation, followed by chemical oxidation). More than 90% of the commercial product is currently made from this cultivated form, but several wild forms were once wild-crafted on a small scale (or added to the red type to improve the fermentation process and/or the flavour). This information was supplied to B-EvW by local farmers (Les Abrahams of “Little Boys Kraal” and Frans du Plessis of “Aggenbachskraal”). The variation in the wild tea types in the northern part of the distribution range has been described by Malgas et al. (2010). The Wupperthal and Nieuwoudtville types are still wild-harvested as speciality products for niche markets.

Rooibos tea is made by cutting the young stems and needle-shaped leaves of the plant into 2–3 (–5) mm long sections (using a modified silage cutter), after which they are bruised and

* Corresponding author. Phone: +27 115592412; fax: +27 115592411.

E-mail address: bevanwyk@uj.ac.za (B.-E. Van Wyk).

Table 1
Leaf diameter as measured in 18 tea samples made from the seven main types of rooibos tea (*Aspalathus linearis*).
Tea types and locality details as in Van Heerden et al. (2003).

Specimen number	Tea type	Locality	Mean leaf diameter ± standard error (n = 30), μm
1	Red type	Clanwilliam	968 ± 32
2		Pakhuis Pass	771 ± 22
3		Boskloof	795 ± 33
4		Nardouwsberg	703 ± 13
5		Kriedouw	1005 ± 28
6	Southern resprouter	Kriedouw	811 ± 31
7		Elandskloof	1179 ± 37
8	Grey resprouter	Kriedouw, population 1	962 ± 18
9		Kriedouw, population 2	802 ± 22
10		Duiwelskop	1078 ± 35
11	Northern resprouter	Pakhuis Pass	816 ± 16
12		Gifberg	834 ± 19
13	Black type	Nieuwoudtville	795 ± 16
14		Piekenierskloof	929 ± 38
15	Tree type	Duiwelskop	794 ± 18
16		Kriedouw	1021 ± 24
17	Wupperthal type	Biedouw	871 ± 20
18		Eselbank	948 ± 24

moistened to enhance the natural enzymatic oxidation process that turns the leaves from green to a rich red-brown colour. This process also degrades most of the flavonoid content (mainly aspalathin) that was shown to occur throughout the green, unfermented leaf (Baranska et al., 2006). Green rooibos is a new innovation — the oxidation process is stopped, resulting in high levels of aspalathin (the main phenolic compound in rooibos tea). Rooibos tea has become an important herbal tea and health drink (Morton, 1982; Van der Walt and Machado, 1992; Joubert and De Beer, 2011), with an annual production of up to 18,000 tonnes. In 2003, exports to Germany alone exceeded the local consumption in South Africa. The product also has numerous medicinal benefits (e.g. antioxidant, anti-inflammatory, anti-carcinogenic, hypoglycaemic and photoprotective effects), as outlined in the excellent review by Joubert and De Beer (2011).

The main chemical compounds of rooibos tea, and the effects of oxidation on the phenolic constituents, have been well studied (Rabe et al., 1994; Joubert, 1996; Marais et al., 2000; Joubert and De Beer, 2011, and references cited therein), but very limited information is available on the morphological and anatomical characteristics of the product. The aim of our

study was therefore to provide detailed anatomical descriptions of the stems and leaves of *A. linearis* and to evaluate the pharmacognostic value of anatomical characters.

2. Materials and methods

Commercial product was studied in the dry condition and after softening in hot water. Four samples of *A. linearis* (two from reseeding and two from resprouting populations) were used for anatomical analysis (voucher specimens all in JRAU): 1, commercial Red type (reseeded; Citrusdal, Aggenbagskraal, KK51-11); 2, Black type (reseeded; Citrusdal, Aggenbagskraal, KK50-11); 3, Grey type (resprouter; Citrusdal, Elandskloof, KK48-11); and 4, Decumbent type (resprouter; Pakhuis Pass, Clanwilliam, KK56-11). The material was collected in May 2011. Leaf diameters were measured using 18 tea samples as listed in Table 1. Details about the tea types, localities and voucher specimens were presented by Van Heerden et al. (2003) and are not repeated here. These samples were carefully subjected to the standard commercial production treatment, which includes a fermentation period of 12 h. Young shoots and leaves, as well as pieces of old thick trunks were fixed in FAA (Johansen, 1940). Transverse sections of leaves were made using a freezing microtome (Leitz Lauda Kryomat 1700) to retain the natural yellow colour of the oxidised compounds in the samples and also with an ultra-microtome (Porter-Blum Sorvall MT-1) after embedding in glycol methacrylate (GMA) and staining with toluidine blue and Schiff's reagent according to the method of Feder and O'Brien (1968). To study the stems, three different portions (20–100 mm long) were cut: (1) from branch tips without a visible periderm; (2) from lower parts of the stem where the periderm was starting to form and (3) from thicker stems with mature bark having a more or less thick periderm and a considerable amount of wood. Transverse, radial and tangential sections were prepared with the freezing microtome and stained with a 1:1 alcian blue/safranin mixture (Jansen et al., 2004). Pieces of bark were also macerated in Jeffrey's solution (Johansen, 1940). Photographs and measurements were made using an Olympus ColorView Soft Imaging System and the Olympus Analysis Imaging Solutions (OASIS) programme.

3. Results

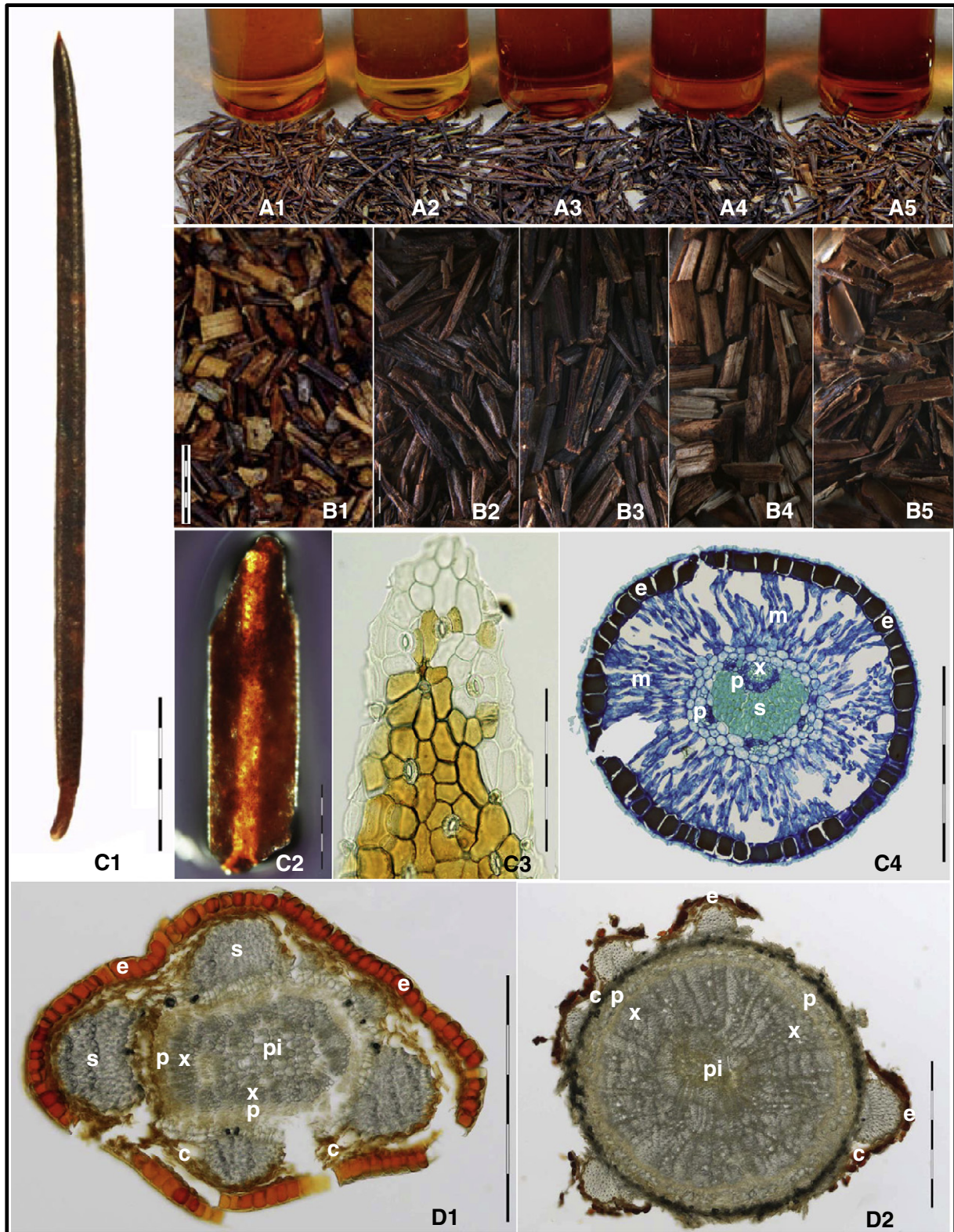
3.1. Variation in colour

The colour of the main commercial type of rooibos tea (both the product and the decoctions or infusions derived from it) is

Fig. 1. Variation in the morphology, anatomy and colour of rooibos tea (*Aspalathus linearis*). A1–5, examples of the main types of rooibos tea (A1, Red type — Rocklands reseeded; A2, Red-brown type — Nieuwoudtville reseeded; A3, Grey type — Elandskloof resprouter; A4, Black type — Piekenierskloof reseeded; A5, Wupperthal type — Biedouw reseeded); B1–5, examples of commercial tea (B1, commercial red rooibos tea; B2–5, various components of rooibos tea separated: B2, leaves; B3, stems; B4, wood fragments; B5, bark fragments); C1–4, leaf structure and anatomy (C1, general view of leaf; C2, leaf fragment under polarised light to show the fibrous vascular bundle; C3, leaf surface showing epidermal cells with anomicytic stomata (note the yellow colour of the phenolic compounds); C4, transverse section of a leaf showing the phenolic compounds in the epidermis — note the fibrous main xylem bundle and ring of small bundles; D1–2, transverse sections of stems; D1, thick section of younger stem showing the epidermal cells with phenolic compounds in the epidermis; D2, thick section of older stem with a continuous ring of periderm initiated under the primary phloem fibres and the remains of the epidermis. c = cortex; e = epidermis; m = mesophyll; p = phloem; pi = pith; x = xylem; s = sclerenchyma; scale bars: B1–5, C1 = 5 mm; C2 = 1 mm; C3 = 0.2 mm; C4, D1–2 = 0.5 mm.

typically a bright reddish brown (Fig. 1, A1), but the Wupperthal type is quite similar (Fig. 1, A5). Fermented leaves of the wild forms vary in colour from reddish-brown (Fig. 1, A2) to grey (Fig. 1, A3) or black (Fig. 1, A4) but the infusions or decoctions

made thereof are similar to that of the commercial red form (Fig. 1, A1 vs A2–5). Young shoots and leaves are dull green in green rooibos tea but yellowish to reddish-brown with a lustrous surface in normal “fermented” rooibos tea (Fig. 1, B1).



3.2. Stem and leaf morphology of the commercial Red type

Rooibos tea predominantly consists of fragments of leaves, stem tips, wood and bark, about 2 to 3 (up to 5) mm long (Fig. 1, B2–5). The leaves (originally terete and needle-shaped — Fig. 1, C1) are 0.5 to 1.2 (up to 1.5) mm in diameter (mean values vary from 703 to 1179 μm , as shown in Table 1) and are commonly flattened (due to the production process that involves cutting and bruising). Young stems are somewhat ribbed (more strongly so when older) and the cut pieces are very similar to those of the leaf but are much firmer in texture. Wood fragments are white but may become reddish during processing because the woody stems absorb oxidised phenolic compounds from the leaves (extracted when water is added during fermentation). Bark pieces are flat, dark reddish-brown on one surface and often white on the other. The ratio between leaf, stem, wood and bark is exceptionally variable but in good quality tea, the leaves and stems are usually in equal proportions and together represent more than half (often 60% or more) of the total weight of the product. The remaining 40% comprises wood and bark, in a ratio of typically about 3:1.

3.3. Leaf anatomy

Leaves are round in transverse section (Fig. 1, C4). The epidermis consists of a single row of square cells containing yellowish-brown inclusions (Fig. 1, C4). The cuticle is thick (6–12 μm), being slightly thicker than the outer periclinal cell wall (3–10 μm). Uni- or bicellular trichomes rarely occur on the epidermal surface. Stomata are anomocytic, evenly distributed on the leaf surface (Fig. 1, C3), and usually level with the epidermis or slightly sunken. They are clearly visible in polarised light. The mesophyll is composed of two or three layers of palisade cells having a length to width ratio of between three and six; spongy tissue is absent. The vascular tissue of the midrib consists of a large, somewhat circular bundle (five or six xylem cells in diameter) with additional smaller bundles (rarely lacking phloem) arranged more or less in a ring with the phloem orientated inwardly (Fig. 1, C4). The central well-developed sclerenchymatous tissue of the main vascular bundle is composed of fibres with very thick walls which almost completely close the lumina. This central bundle is clearly visible in polarised light (Fig. 1, C2).

3.4. Stem anatomy

The epidermis consists of a single layer of cells which are radially elongate and include yellowish brown contents (Fig. 1, D1). Uni- or bicellular trichomes are rarely present. The cortex is composed of three to six layers of isodiametric to radially-flattened, thin-walled cells. Chloroplasts are present in the cortical parenchyma cells. Vascular bundles are collateral. Primary phloem fibres have very thick walls and commonly occur in large groups of 70 to 150. The pith is round, oval or slightly stellate and composed of parenchyma cells.

Secondary growth occurs early in development. The initiation of the first-formed periderm is under the groups of primary phloem fibres (Fig. 1, D2). Phellem is composed of five to 15 layers of radially-flattened cells with thickened outer walls; phelloderm comprises two or three layers of radially-flattened, thin-walled cells. Subsequent periderms are initiated in the outer region of the cortical parenchyma and cut scales. 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. Dilatation of secondary phloem is radial. Sieve plates are compound and are located on the oblique cross walls. Conspicuous sieve areas are found on the lateral walls. Wood is diffuse-porous. Growth rings are distinct to faint. Vessels are numerous, narrow, angular in outline, and occur in clusters, radial multiples or are solitary. Fibres have very thick walls. Rays are narrow and uniseriate.

4. Discussion and conclusions

The recent monograph by Hiller and Löw (2009) gives some information on the general morphology of the stems, leaves and the product, as well as a brief description and illustration of the epidermis. Our more detailed anatomical analysis has not only provided additional characters that can be used to identify the product, but has also led to a better understanding of the structural features of the leaf and stem that contribute to product variability and product quality.

The reddish brown oxidation products are highly localised and occur only in the epidermis of leaves and young stems. These two components make up about 55% of the commercial product. Secondary phloem and xylem are colourless (i.e. without the brown oxidation products), yet constitute about 45% of the product. This explains the benefits of using a reduced mesh size of the sieves, as required by some clients, because a larger percentage of xylem would be removed from the final product. The coarse (woody) fraction was once discarded, but has in recent years become valuable for the production of ice teas and extracts.

Wild forms of rooibos tea are anatomically very similar to the commercial Red type despite obvious differences in colour. As is evident from Table 1, leaf diameter is too variable to be useful as a diagnostic character for different tea types. We could also find no morphological or anatomical characters to distinguish between the various tea types so that the highly variable phenolic profiles (Van Heerden et al., 2003) present the most practical way of identifying the provenance of a particular tea sample. More work is needed to explore the phenolic variability in the commercial product and in the numerous wild tea types. The colour of the fermented product is an important character, ranging from bright red-brown to various shades of brown, grey and black. The commercial use of grey and black types was discontinued in 1966 (Rooibos Tea Control Board 12th Annual Report, 1967) because of poor quality (colour and/or taste) but with standardisation, some wild types with excellent flavour may be overlooked. Anatomical features of potential value to distinguish between rooibos tea

and other herbal teas or adulterants include the following: (1) the terete leaf segments with bright yellowish brown epidermal cells; (2) the morphologically similar (but much firmer) stem sections, also with yellowish brown epidermal cells; (3) the thick cuticle; (4) the anomocytic stomata; (5) the ring of small vascular bundles around the main bundle in the leaf and (6) the highly sclerified main vascular bundle of the leaf (clearly visible under polarised light).

Acknowledgements

The authors thank the National Research Foundation and University of Johannesburg for funding.

References

- Baranska, M., Schulz, H., Joubert, E., Manley, M., 2006. In situ flavonoid analysis by FT-Raman spectroscopy: identification, distribution, and quantification of aspalathin in green rooibos (*Aspalathus linearis*). *Analytical Chemistry* 78, 7716–7721.
- Dahlgren, R., 1968. Revision of the genus *Aspalathus*. II. The species with ericoid and pinoid leaflets. 7. Subgenus *Nortieria*. With remarks on rooibos tea cultivation. *Botaniska Notiser* 121, 165–208.
- Dahlgren, R., 1988. *Crotalariaeae (Aspalathus)*. *Flora of Southern Africa* 16, 84–90.
- Feder, N., O'Brien, T.P., 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* 55, 123–142.
- Hawkins, H.-J., Malgas, R., Biénabe, E., 2011. Ecotypes of wild rooibos (*Aspalathus linearis* (Burm. F) Dahlg., Fabaceae) are ecologically distinct. *South African Journal of Botany* 77, 360–370.
- Hiller, K., Löw, D., 2009. *Aspalathus linearis herba*. In: Wichtl, M. (Ed.), *Teedrogen und Phytopharmaka*. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, pp. 99–101.
- Jansen, S., Choat, B., Vinckier, S., Lens, F., Schols, P., Smets, E., 2004. Intervascular pit membranes with a torus in the wood of *Ulmus* (Ulmaceae) and related genera. *New Phytologist* 163, 51–59.
- Johansen, D.A., 1940. *Plant Microtechnique*. McGraw-Hill, New York.
- Joubert, E., 1996. HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. *Food Chemistry* 55, 403–411.
- Joubert, E., De Beer, D., 2011. Rooibos (*Aspalathus linearis*) beyond the farm gate: from herbal tea to potential phytopharmaceutical. *South African Journal of Botany* 77, 869–886.
- Malgas, R.R., Potts, A.J., Oetlé, N.M., Koelle, B., Todd, S.W., Verboom, G.A., Hoffman, M.T., 2010. Distribution, quantitative morphological variation and preliminary molecular analysis of different growth forms of wild rooibos (*Aspalathus linearis*) in the northern Cederberg and on the Bokkeveld Plateau. *South African Journal of Botany* 76, 72–81.
- Marais, C., Janse van Rensburg, W., Ferreira, D., Steenkamp, J.A., 2000. (S)- and (R)-Eriodictyol-6-C- β -d-glucopyranoside, novel keys to the fermentation of rooibos tea (*Aspalathus linearis*). *Phytochemistry* 55, 43–49.
- Morton, J.F., 1982. Rooibos tea, *Aspalathus linearis*, a caffeineless low-tannin beverage. *Economic Botany* 37, 164–173.
- Rabe, C., Steenkamp, J.A., Joubert, E., Burger, J.F.W., Ferreira, D., 1994. Phenolic metabolites from rooibos tea (*Aspalathus linearis*). *Phytochemistry* 35, 1559–1565.
- Schutte, A.L., Vlok, J.H.J., Van Wyk, B.-E., 1995. Fire-survival strategy — a character of taxonomic, ecological and evolutionary importance in fynbos legumes. *Plant Systematics and Evolution* 195, 243–259.
- Van der Bank, M., Van Wyk, B.-E., Van der Bank, H., 1995. Biochemical genetic variation in four wild populations of *Aspalathus linearis* (rooibos tea). *Biochemical Systematics and Ecology* 23, 257–262.
- Van der Bank, M., Van der Bank, H., Van Wyk, B.-E., 1999. Evolution of sprouting versus seeding in *Aspalathus linearis*. *Plant Systematics and Evolution* 219, 27–38.
- Van der Walt, A., Machado, R., 1992. *New Marketing Success Stories*. Southern Book Publishers, Halfway House.
- Van Heerden, F.R., Van Wyk, B.-E., Viljoen, A.M., Steenkamp, P.A., 2003. Phenolic variation in wild populations of *Aspalathus linearis* (rooibos tea). *Biochemical Systematics and Ecology* 31, 885–895.