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Short communication

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

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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.

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 Crotalarieae), 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

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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	Grey resprouter	Elandskloof	1179 ± 37
8		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		Nieuwoudtville	795 ± 16
14	Black type	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 (reseeder; Citrusdal, Aggenbagskraal, KK51-11); 2, Black type (reseeder; 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 reseeder; A2, Red–brown type — Nieuwoudtville reseeder; A3, Grey type — Elandskloof resprouter; A4, Black type — Piekenierskloof reseeder; A5, Wupperthal type — Biedouw reseeder); 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 anomocytic 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.