Pergamon

Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochemical Systematics and Ecology 31 (2003) 885–895

biochemical systematics and ecology

www.elsevier.com/locate/biochemsyseco

Phenolic variation in wild populations of *Aspalathus linearis* (rooibos tea)

F.R. van Heerden [a], B.-E. van Wyk [b,*], A.M. Viljoen [b,1], P.A. Steenkamp [a,2]

[a] *Department of Chemistry & Biochemistry, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, South Africa*

[b] *Department of Botany, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, South Africa*

Received 10 December 2002; accepted 12 December 2002

Abstract

Rooibos tea, a herbal tea derived from *Aspalathus linearis* (Fabaceae—tribe Crotalarieae) has become increasingly popular as a health drink. The beneficial properties are partly ascribed to the phenolic constituents in the plant, which are enzymatically modified during processing. Within the species, distinct geographical forms can be recognised, differing in habit, fire-survival strategy, vegetative and reproductive morphology, enzyme patterns and flavonoids. Several phenolic compounds are known to occur in *A. linearis*. The main constituent of the commercial tea type (also known as the Rocklands type or the red tea type) is the dihydrochalcone aspalathin, but the presence of other flavonoids such as nothofagin, orientin, isoorientin, vitexin, rutin and isoquercetrin has been reported. These compounds are found in the processed product, as well as the dried leaves. The relative quantities of phenolic compounds show large qualitative and quantitative differences between populations and provenances of the wild tea types. Within populations, however, the patterns are remarkably uniform. Aspalathin was found to be the main compound in processed ("fermented") tea and also in dried leaves of several tea types. The compound is absent in some wild tea populations, where other flavonoids occur as the main phenolics, notably orientin, isoorientin and rutin. Rutin is invariably the main compound in *Aspalathus pendula*, a close relative of *A. linearis* that is rarely used to make

* Corresponding author. Tel.: +27-11-489-2412; fax: +27-11-489-2411.
E-mail address: bevw@na.rau.ac.za (B.-E. van Wyk).

[1] Present address: Department of Pharmacy, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa.

[2] Present address: Forensic Chemistry Laboratory, Department of Health, P.O. Box 1080, Johannesburg 2000, South Africa.

doi:10.1016/S0305-1978(03)00084-X

tea. An overview of natural variation in phenolic compounds of the main wild tea types is presented.

Keywords: *Aspalathus linearis*; Rooibos tea; *A. pendula*; Fabaceae; Phenolics; Infraspecific chemical variation

1. Introduction

Aspalathus linearis (Burm.f.) Dahlg. is commercially cultivated on a large scale for the production of rooibos tea, a traditional herbal health drink (Morton, 1982; Van der Walt and Machado, 1992). A closely related species, *A. pendula* Dahlg., as well as several wild types of *A. linearis* are sometimes used to make tea on a non-commercial scale. The genus *Aspalathus* (Fabaceae–tribe Crotalarieae) comprises about 278 species and is endemic to South Africa. The species are concentrated in the Cape region, with a few spreading to southern KwaZulu-Natal. *Aspalathus linearis* and *A. pendula* belong to a small but very distinct group of species with simple, needle-like leaves (phyllodes). This group was first treated as a subgenus by Dahlgren (1968), [formally described as subgenus *Nortieria* Dahlg.] but in a comprehensive revision (Dahlgren, 1988), the species were treated as an informal group, *Lebeckiiformes*. Some subspecies were combined, while others were given specific status. A summary of the two classification systems and the correct nomenclature of all the taxa are given in Table 1.

Aspalathus linearis is exceptionally polymorphic and distinct geographical forms can be recognised (Dahlgren, 1968, 1988). Each of these forms differs in habit, fire-survival strategy, vegetative and reproductive morphology, enzyme patterns and flavonoids (Dahlgren, 1968; Van der Bank et al., 1995, 1999). No satisfactory infraspecific classification system has yet been devised.

Several phenolic compounds are known to occur in *A. linearis*. The main constituent of the commercial tea type (also known as the Rocklands type or the red tea type) is a unique dihydrochalcone, aspalathin (Koeppen and Roux, 1965), but the

Table 1
Summary of taxonomic and nomenclatural changes: *Aspalathus linearis* and related species

System according to Dahlgren (1968)	**System according to Dahlgren (1988)**
Subgenus *Nortieria* Dahlg. (2 species)	Group *Lebeckiiformes* (4 species)
1a. *A. linearis* (Burm.f.) Dahlg. subsp. *linearis*	1. *A. linearis*
1b. *A. linearis* (Burm.f.) Dahlgr. subsp. *pinifolia* (Marloth) Dahlg.	1. *A. linearis* (no longer distinguished as a subspecies)
1c. *A. linearis* (Burm.f.) Dahlg. subsp. *latipetala* Dahlg.	2. *A. lebeckioides* Dahlg. (raised to rank of species)
2. *A. pendula* Dahlg.	3. *A. pendula* Dahlg.
	4. *A. nudiflora* Harv. (added to group)

presence of several other flavonoids such as nothofagin, orientin, isoorientin, vitexin, rutin and isoquercetrin has been reported (Rabe et al., 1994; Joubert, 1996). These studies all focussed on the commercial tea type, and nothing appears to be have been published on the phenolic compounds of the various wild tea types. Dahlgren (1968) used two-dimensional thin layer chromatography to show the flavonoid patterns of the main tea types, but none of the compounds were identified. The relative quantities of phenolic compounds are to some extent influenced by processing (Joubert, 1996; Marais et al., 2000), but this variation is small when compared to large qualitative and quantitative differences between populations and provenances of the wild tea types derived from *A. linearis*.

The purpose of this paper is to give a broad overview of natural variation in the phenolic compounds of the main wild types of *Aspalathus linearis* and the closely related *A. pendula*. Recent studies on the beneficial effects of rooibos tea have focussed on antioxidant and antimutagenic properties (Von Gadow et al., 1997a, 1997b; Marnewick et al., 2000; Standley et al., 2001). It is conceivable that these activities will be influenced by the nature and relative quantities of flavonoids present in the tea. The large quantitative and qualitative variation described below may eventually be of value in the selection and breeding of superior tea types.

2. Materials and methods

Leaf material was collected from ten individual plants of each of 18 populations of *Aspalathus linearis* and two populations of *A. pendula*. Herbarium voucher specimens for each of the populations sampled, as well as accurate locality data recorded by GPS, are listed in Table 3. Three samples (rarely one or two samples only, in one case six) of each population (1.0 g rapidly dried, fresh leaves per sample) were ground to a fine powder and extracted with methanol. Extracts were filtered through celite and prepared for TLC and HPLC analysis by solid phase extraction on C_{18} columns (Machery-Nagel Chromabond®, 6 ml/1000 mg). Samples were taken to dryness on a rotary evaporator, redissolved in methanol, mixed with an equal volume of water and subjected to HPLC analysis. The HPLC system for routine analyses comprised an IB-Sil column (C18, 5 μm, 250×4.6 mm, flow rate 1 ml min^{-1}, 20 μl sample loop). The solvent system was a 30–100% linear gradient of methanol in 1% acetic acid-water over 20 min. Detection was by diode array detector, using two channels (A set at 280 ± 40 nm, B set at 330 ± 70 nm). The presence of aspalathin, nothofagin, orientin, iso-orientin and rutin was confirmed in two samples by co-injection with authentic standards (see Joubert, 1996). The identity of some of the main flavonoids was confirmed by LC-MS. Five selected samples were subjected to HPLC-MS studies using the following system: Waters 2690 HPLC system (Phenomenex Aqua C_{18} column, 250×2.1 mm) equipped with both a 996 photodiode array (PDA) detector and a Thermabeam mass selective detector (TMD) [electron impact (EI) mode], or alternatively a Z spray mass selective detector (ZMD) [electrospray mode]. The TMD detector was operated in electron impact mode with the ionizer at 70 eV, with a gain of 10 and scanning a mass range of 50–550 amu. The

Table 3
Summary of main phenolic compounds in 57 individual plants from 20 populations of *Aspalathus linearis* and *A. pendula*. Accurate localities are provided for most wild types of *A. linearis* (less accurate for some rare taxa and forms). Voucher specimen numbers refer to the collections of B-E van Wyk, all housed in the Rand Afrikaans University Herbarium (JRAU). (aspa = aspalathin, orie = orientin, isoo = isoorientin, noth = nothofagin, ruti = rutin; X1 = unknown flavanone/dihydro-chalcone, Rt 7.31; X2 = unknown flavone, Rt 11.6; X3 = unknown flavone, Rt 14.12; X4 = unknown flavanone/dihydrochalchone, Rt 15.15; X5, 3-O-rutinoside of kaempferol, possibly nicotiflorin, Rt 18.66; under OTHER, flavonoids with unique occurrences in only one population or sample are listed. tr = less than 10% of total absorbance, + = 10 to 20%, ++ = 20 to 50%, +++ = above 50%

Species, biotypes & populations	**Voucher specimens**	**Main phenolic compounds**										
		aspa	orie	isoo	noth	ruti	X1	X2	X3	X4	X5	OTHER
Aspalathus pendula												
Piquetberg												
(33° 30′ S, 18° 30′ E)	BEVW 3619											
plant 1		–	–	–	–	+++	–	–	–	–	tr	
plant 2		–	–	–	–	+++	–	–	–	–	tr	
plant 3		–	–	–	–	+++	–	–	–	–	tr	
Aggenbachskraal												
(32° 18′ S, 18° 51′ E)	BEVW 3628											
plant 1		–	–	–	–	+++	–	–	–	–	tr	
plant 2		–	–	–	–	+++	–	–	–	–	tr	
plant 3		–	–	–	–	+++	–	–	–	–	tr	
Aspalathus linearis												
1. Southern resprouter												
Franschhoek Pass												
(33° 45′ S, 19° 00′ E)	BEVW 2998											
plant 1		–	++	+++	–	–	–	–	–	–	–	
Romansrivier												
(33° 05′ S, 19° 00′ E)	BEVW 3122											
plant 1		–	++	+++	–	–	–	–	–	–	–	

(*continued on next page*)

Table 3 (*continued*)

Species, biotypes & populations	**Voucher specimens**	**Main phenolic compounds**										
		aspa	orie	isoo	noth	ruti	X1	X2	X3	X4	X5	OTHER
Kriedouw												
(32° 22′ 40″ S, 19° 01′ 25″ E)	BEVW 3624											
plant 1		–	–	–	–	+++	–	–	–	–	+	
plant 2		tr	–	–	–	+++	–	–	–	–	+	
plant 3		tr	–	–	–	+++	–	–	–	–	+	
2. Grey resprouter												
Elandskloof												
(32° 37′ 09″ S, 19° 03′ 33″ E)	BEVW 3621											
plant 1		–	+	+	–	–	+++	–	–	–	–	
plant 2		–	++	++	–	–	+	–	–	–	–	
plant 3		–	+++	++	–	–	–	–	–	–	–	
Kriedouw												
(32° 22′ 40″ S, 19° 01′ 25″ E)	BEVW 3624d											
plant 1		+++	tr	tr	+	–	–	–	–	–	–	
plant 2		+++	tr	tr	tr	–	–	–	–	–	–	
plant 3		+++	tr	tr	tr	–	–	–	–	–	–	
plant 4		+++	tr	tr	tr	–	–	–	–	–	–	
plant 5		+++	tr	tr	tr	–	–	–	–	–	–	
plant 6		+++	tr	tr	tr	–	–	–	–	–	–	
Duiwelskop												Rt: 15.82
(32° 28′ 03″ S, 19° 01′ 17″ E)	BEVW 3622											
plant 1		+++	tr	tr	tr	tr	tr	–	–	–	–	++
plant 2		+++	tr	tr	tr	tr	tr	–	–	–	–	+
plant 3		+++	tr	tr	tr	tr	tr	–	–	–	–	+
3. Northern resprouter												
Pakhuis Pass												Rt: 11.93
(32° 08′ 19″ S, 18° 59′ 04″ E)	BEVW 3617											
plant 1		+++	tr	tr	tr	tr	tr	–	tr	+	–	+
plant 2		++	tr	tr	tr	tr	tr	–	+	+	–	+

Table 3 (*continued*)

Species, biotypes & populations	Voucher specimens	Main phenolic compounds										
plant 3		++	tr	tr	tr	tr	tr	–	+	+	–	+
Gifberg												Rt: 9.70
(31° 45′ 58″ S, 18° 46′ 08″ E)	BEVW 3613											
plant 1		–	tr	+	–	–	–	–	+	–	+	+++
plant 2		–	tr	+	–	–	–	–	+	–	+	+++
plant 3		–	tr	+	–	–	–	–	+	–	+	+++
Nieuwoudtville												
(31° 34′ 58″ S, 19° 09′ 34″ E)	BEVW 3612											
plant 1		+++	tr	+	tr	–	–	–	+	–	+	
plant 2		+++	tr	+	tr	–	–	–	+	–	+	
plant 3		+++	tr	+	tr	–	–	–	+	–	+	
4. Red type												
Rocklands												
(32° 08′ 19″ S, 18° 59′ 08″ E)	BEVW 3614											
plant 1		+	tr	tr	–	–	tr	–	–	–	–	
plant 2		+++	tr	tr	tr	tr	tr	–	–	–	–	
plant 3		+++	tr	tr	tr	tr	tr	–	–	–	–	
Boskloof												Rt: 12.07
(32° 15′ 09″ S, 19° 01′ 38″ E)	BEVW 3618											
plant 1		+	tr	tr	–	–	–	–	+	++	–	++
plant 2		tr	tr	tr	–	–	–	–	+	++	–	++
plant 3		+	tr	tr	–	–	–	–	+	++	–	++
Nardouwsberg												
(31° 54′ 01″ S, 18° 49′ 14″ E)	BEVW 3625											
plant 1		tr?	+++	++	tr	–	–	–	–	–	–	
plant 2		–	+++	++	tr	–	–	–	–	–	–	
Kriedouw												
(32° 19′ 35″ S, 18° 58′ 30″ E)	BEVW 3624a											
plant 1		++	tr	tr	tr	tr	tr	–	++	–	–	
plant 2		+++	tr	tr	tr	tr	tr	–	+	–	–	
plant 3		++	tr	tr	tr	tr	tr	–	++	–	–	

Table 3 (*continued*)

Species, biotypes & populations	Voucher specimens	Main phenolic compounds										
5. Black type												
Piekenierskloof												
(32° 36′ 22″ S, 18° 58′ 41″ E)	BEVW 3620											Rt: 15.90
plant 1		–	++	++	–	–	–	–	–	–	–	++
plant 2		–	++	++	–	–	–	–	–	–	–	++
plant 3		–	++	++	–	–	–	–	–	–	–	++
6. Tree type												
Duiwelskop												
(32° 29′ 24″ S, 19° 03′ 15″ E)	BEVW 3623											
plant 1		+++	tr	tr	tr	+	–	–	+	–	–	
plant 2		+++	tr	tr	tr	tr	–	–	+	–	–	
plant 3		+++	tr	tr	tr	tr	–	–	+	–	–	
Kriedouw												
(32° 19′ 36″ S, 18° 58′ 20″ E)	BEVW 3624b											
plant 1		+++	tr	tr	tr	+	tr	–	+	–	–	
plant 2		+++	tr	tr	tr	tr	tr	–	tr	–	–	
plant 3		+++	tr	tr	tr	tr	tr	–	tr	–	–	
7. Wupperthal type (“spp. *pinifolia*”)												
Biedouw												
(32° 10′ 50″ S, 19° 09′ 26″ E)	BEVW 3626											
plant 1		++	tr	tr	tr	tr	++	tr	tr	–	–	
plant 2		++	tr	tr	tr	tr	++	tr	tr	–	–	
plant 3		++	tr	tr	tr	tr	++	tr	tr	–	–	
Eselbank												
(32° 21′ 33″ S, 19° 13′ 54″ E)	BEVW 3627											
plant 1		++	tr	tr	tr	tr	tr	tr	–	–	–	
plant 2		++	tr	tr	tr	tr	tr	tr	–	–	–	

flow rate of the HPLC was 0.2 ml/min and the gas flow through the nebulizer 30 L/h. The nebulizer temperature was 80 °C, the expansion region 90 °C and the source temperature 225 °C. No flow splitter was used. The ZMD was operated in positive mode with no flow splitting and an HPLC flow rate of 0.2 ml/min.

Observations on the morphology and fire-survival strategy of *Aspalathus* populations were made by one of us (BEVW) during periodic field studies over many years (since 1983 to the present).

3. Results and discussion

3.1. Morphological variation and infraspecific taxonomy

Aspalathus linearis is an extremely variable species complex with numerous populations or biotypes (Dahlgren, 1968). These populations or biotypes can be divided into two basic groups according to their fire-survival strategy (Schutte et al., 1995): 1, Reseeders, which are killed by fire and re-establish through seeds—plants typically have single stems and an erect growth form; 2, Resprouters, which are not killed by fire but resprout again from a subterranean lignotuber—they have multiple stems and often a prostrate growth form. Van der Bank et al. (1995, 1999) have shown that there are genetic differences between seeding and sprouting populations and that sprouting may be a derived character that has evolved to ensure survival in a fireprone environment. The closely related *A. pendula* is a reseeding species with a slender main trunk of up to 5 m tall. A similar habit is seen in the arborescent form of *A. linearis*, which is also a reseeder. This so-called tree type has glaucous leaves, similar to the grey resprouters from the Citrusdal area. Flowers vary markedly in size, shape and colour from one population to the next. The Wupperthal form of the species (previously subsp. *pinifolia*), for example, has a pronounced awn at the tip of the maroon-coloured keel petals. The commercial (cultivated) form of the species is a reseeder with yellow flowers, bright green leaves and an erect, densely branched growth form. Several populations have the flowers partly or completely reddish to maroon or mauve.

A preliminary infraspecific classification system for the various biotypes of *A. linearis*, based on fire-survival strategy and overall morphology, is given in Table 2.

3.2. Chemical variation in tea types

Most people are familiar with the commercial red tea type, but there are several other (wild) types that differ markedly in texture, colour and aroma. The main ones are the red, grey, black, Wupperthal and yellow types (the last-mentioned is made from *A. pendula*). Within each of these basic types there are various subtypes (populations) with subtle differences in colour and aroma, not unlike the range of variation found in wine cultivars. Some of these tea types have distinctive phenolic profiles. The dehydrochalcone aspalathin is the main phenolic compound in the red tea type, and occurs as the main constituent in many populations of *A. linearis*.

Table 2
Suggested infraspecific classification system for *Aspalathus linearis*. Note that there are significant morphological, genetic and chemical differences amongst populations within each of the basic biotypes

Resprouting types	Reseeding types
1. Southern resprouter Small, 0.2–0.3 m, erect to procumbent; leaves thin, bright green, often pubescent; flowers small, yellow. Populations: Franschhoek Pass Romansrivier Kriedouw	**4. Red type** Medium-sized, 0.6–2 m, erect, dense; leaves bright green; flowers medium-sized, yellow. Populations: Rocklands Boskloof Nardouwsberg Kriedouw
2. Grey resprouter Medium-sized, 0.3–0.8 m, erect to procumbent; leaves usually thick, glaucous, glabrous; flowers medium-sized, partly yellow, partly reddish. Populations:Elandskloof Kriedouw Duiwelskop	**5. Black type** Medium-sized, 0.6–2 m, erect, sparse; leaves medium-sized, greyish-green; flowers medium-sized, partly yellow, partly reddish. Populations: Piekenierskloof
3. Northern resprouter Medium-sized, 0.3–0.5 m high, up to 0.8 m wide, procumbent; leaves usually medium-sized, green to slightly glaucous, glabrous; flowers small to medium-sized, partly to predominantly reddish-purple. Populations: Pakhuis Pass Gifberg Nieuwoudtville	**6. Tree type** Large, 2–4 m high, erect, sparse, single-stemmed; leaves thick, glaucous; flowers medium-sized, partly yellow, partly reddish. Populations:Duiwelskop Kriedouw **7. Wupperthal type ("ssp. *pinifolia*")** Medium-sized, 0.5–1.5 m high, dense, erect; leaves thin, bright green; flowers small, yellow and maroon-red, with an apiculate carina. Populations: Biedouw Eselbank

Several other flavonoids are present, including unique occurrences in some populations. A summary of the main compounds in 57 plants from 20 populations of *A. linearis* and *A. pendula* is given in Table 3. It can be seen that a high concentration of rutin, combined with a small amount of a compound tentatively identified as nicotiflorine and a total absence of aspalathin or any other flavonoids, are characteristic of *A. pendula*. Only the southern resprouter from Kriedouw has (unexpectedly) the same profile, although trace amounts of aspalathin appear to be present here. In general, *A. linearis* has aspalathin as a major phenolic compound in most populations, but occasionally this is replaced by orientin and isoorientin. The remarkable uniformity within populations is noteworthy—variation, if any, is quantitative only. Most of the variation is at the level of population – the various tea types show large differences, and the phenolic profiles are rarely of diagnostic value to differentiate between tea types. There are no clearcut differences between resprouters and reseeders, and also not between black, red, grey and tree types.

The two southern resprouting populations (Franschhoek Pass and Romansrivier) appear to be quite distinct. The combination of aspalathin and the unknown flavanone/dihydrochalcone X1 appears to be diagnostic for the Wupperthal type. The farm Kriedouw near Citrusdal has no fewer than four distinct types and appears to be a centre of diversity for the species. What is quite remarkable is that the morphological differences between these types are maintained even though they sometimes occur in close proximity. During several years of field observations, one of us (BEVW) has only once seen putative hybrids (three plants) in the narrow zone between two populations of the red and tree types.

Some populations have unique compounds not found in any others. Examples are the black type (Piekenierskloof), the Gifberg population and the Boskloof population. Boskloof has been suggested as one of the possible original seed sources of the cultivated red tea type, but judging from the HPLC results this seems unlikely. The Rocklands population has the same phenolic profile as the commercial type, and it should be quite easy to compare the commercial type with other wild red types, because the phenolic diversity is unexpectedly high. The Nardouwsberg population seems misplaced amongst populations of the red type as it has the same main compounds as the black type (Piekenierskloof) even though it lacks the distinctive unknown flavanone/dihydrochalchone of the latter. Table 3 shows that most populations have a distinctive "fingerprint" of flavonoids and that further exploration of the total chemical diversity in the species could yield promising results. It may also be worthwhile to isolate and identify the unknown compounds, as these may help to explain more fully the biochemical pathways along which some populations seem to have diversified.

4. Conclusions

The results show that *A. linearis* is a remarkably polymorphic species, not only in terms of morphological and ecological characters but also in its phenolic constituents. Even though aspalathin is the main compound in most of the tea types, several populations have orientin, isoorientin, rutin or various other flavonoids as major constituents. Some tea types and several populations can be identified by unique and distinctive flavonoid profiles. It is clear that growth characteristics and phenolic compounds provide much scope for new crop development and product development in *A. linearis* and *A. pendula*.

Acknowledgements

We wish to thank Mr James van Putten and several producers of rooibos tea for their help and hospitality during many years of field survey work. Dr Elizabeth Joubert helped with HPLC analyses during the early part of this study. Financial support was provided by the National Research Foundation and the Rand Afrikaans University.

References

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. Bot. Notiser 121, 165–208.

Dahlgren, R., 1988. Crotalarieae (*Aspalathus*). Flora of Southern Africa 16 (36), 84–90.

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.

Koeppen, B.H., Roux, D.G., 1965. Aspalathin: a novel *C*-glycosylflavonoid from *Aspalathus linearis*. Tetrahedron Lett 39, 3497–3503.

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.

Marnewick, J.L., Gelderblom, W.C.A., Joubert, E., 2000. An investigation on the antimutagenic properties of South African herbal teas. Mutation Research 471, 157–166.

Morton, J.F., 1982. Rooibos tea, *Aspalathus linearis*, a caffeineless low-tannin beverage. Econ. Bot. 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. Syst. Evol. 195, 243–259.

Standley, L., Winterton, P., Marnewick, J.L., Gelderblom, W.C.A., Joubert, E., Britz, T.J., 2001. Influence of processing stages on antimutagenic and antioxidant potentials of rooibos tea. J. Agric. Food Chem. 49, 114–117.

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). Biochem. Syst. Ecol. 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 Syst. Evol. 219, 27–38.

Van der Walt, A., Machado, R., 1992. New Marketing Success Stories. Southern Book Publishers, Halfway House.

Von Gadow, A., Joubert, E., Hansmann, C.F., 1997a. Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT and BHA. J. Agric. Food Chem. 45, 632–638.

Von Gadow, A., Joubert, E., Hansmann, C.F., 1997b. Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. Food Chemistry 60, 73–77.