Antioxidant Properties of Rooibos (*Aspalathus linearis*) – In Vitro and in Vivo Evidence

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Jeanine L. Marnewick

Contents

Abstract

The South African fynbos plant, *Aspalathus linearis* (Brum.f) Dahlg. (Fabaceae, Tribe Crotalarieae), is traditionally used as a herbal tisane referred to as rooibos or redbush. This plant has claimed medicinal properties based mostly on anecdotal evidence. Rooibos is naturally caffeine free and contains a unique blend of polyphenolic compounds. Based on its in vitro antioxidant potential, a few studies also suggest modulation of oxidative stress/damage by rooibos extracts in experimental animals. More recent studies have examined the bioactivity of rooibos in humans. Together, these factors have contributed to the popularity of

J.L. Marnewick
Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Oxidative Stress Research Centre, Cape Peninsula University of Technology, Bellville, South Africa
e-mail: MarnewickJ@cput.ac.za

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this herbal tea as a health beverage, both locally and internationally. This chapter focuses on the in vitro antioxidant activity of rooibos and discusses recent animal and human studies.

Keywords
Antioxidant capacity • *Aspalathus linearis* • Oxidative stress • Phytochemicals • Rooibos

Introduction

Data from a number of studies during the last decade strongly suggests that a flavonoid-rich diet may offer a strategy for the prevention of lifestyle-related where oxidative stress and inflammation play a role. While evidence from observational and in vitro studies provide us with an inverse association between the intake of dietary antioxidants and the development of disease (Dilis and Trichopoulou 2010) and the recommendation that "high antioxidant" foods should be consumed as part of each meal to prevent postprandial-induced oxidative stress, care should be taken when assuming that an increased plasma antioxidant capacity implies a potential decreased risk of chronic degenerative diseases (Prior et al. 2007). Depending on the type of fruits, vegetables, and beverages (specific teas, red wine, herbal teas) that one consumes, the daily intake of flavonoids can range between 50 and 800 mg (Pietta 2000). Based on the 5-a-day concept and other dietary recommendations, a recent study, using data between 1983 and 2000 determined the average adult South African dietary total antioxidant capacity (TAC) and reported that the South African population only consumes an estimated 50 % of the TAC per day, with beverages being the main contributors (47.8 %) (Louwrens et al. 2009). A report from the USA also confirmed that most adolescents do not consume the US national average of 20 mg flavonoids per day (Beecher 2003).

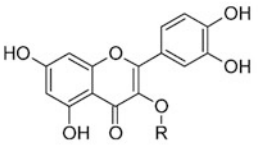
One strategy to ensure increased intakes of flavonoids (and therefore antioxidants) could be to include the addition of tea and/or herbal teas such as rooibos to the diet as a "health boosting" or disease-preventing option. Rooibos research has attracted great interest since the 1990s due to growing anecdotal evidence of its beneficial effects to human health. There is an exponential growth in the number of scientific articles reporting on various biological activities of rooibos, with the past decade yielding the most peer-reviewed articles. There has also been a growing demand for rooibos internationally, with exports increasing from 1,826 t in 1999 to over 6,000 t in 2010, with export of rooibos now exceeding local consumption (data supplied by SA Rooibos Council).

Rooibos flavonoids are unique and differ from those present in *Camellia sinensis* teas. In short, the South African plant, *Aspalathus linearis* (Brum.f) Dahlg. (Fabaceae), is traditionally used in the manufacturing of rooibos (Fig. 181.1) which includes harvesting from January to April (summer months in the Southern hemisphere), heap fermentation of the shredded plant material, sun drying, and

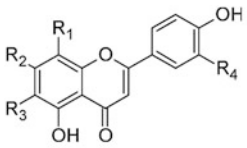
Fig. 181.1 Rooibos plant (*left*) in the Clanwilliam area being hand harvested (*right*) for processing (Courtesy of C Von Metzinger, South Africa)

steam pasteurization before packaging (Joubert and Schulz 2006). More recently, a green/unfermented rooibos has also been produced with a higher antioxidant activity than the traditional/fermented rooibos (Joubert et al. 2008a). In terms of chemical composition, rooibos does not contain caffeine and is considered a low-tannin herbal tea (Reynecke et al. 1949; Blommeart and Steenkamp 1978), rendering this herbal tea a very popular health beverage.

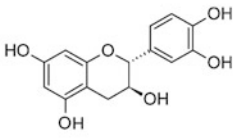
The amount of flavonoids in rooibos can differ depending on factors such as agricultural practices including the fermentation and processing methods used in the manufacturing and genetic variation of the seeds used for propagation (McKay and Blumberg 2007; Joubert et al. 2008b). The main flavonoids (Fig. 181.2) detected in rooibos include the unique *C*–*C*-linked dihydrochalcone glucoside, aspalathin, which is oxidized to the flavanones dihydro-iso-orientin and dihydro-orientin during fermentation; the cyclic dihydrochalcone, aspalalinin; the rare 3-dehydroxy dihydrochalcone glucoside, nothofagin; the *C*-glycosyl flavones orientin, isoorientin, vitexin, and isovitexin; and the flavones hemiphlorin and chrysoeriol, luteolin and luteolin-7-*O*-glucoside, and flavonols quercetin and its *O*-linked glycosides quercetin-3-robinobioside, hyperoside, isoquercitrin, and rutin, with phenolic acids also present (McKay and Blumberg 2007; Joubert et al. 2008b; Marnewick 2009a). These phenolic components are major contributors to the in vitro antioxidant properties of rooibos and are thought to be the foundation for its health-promoting properties (Snijman et al. 2009).



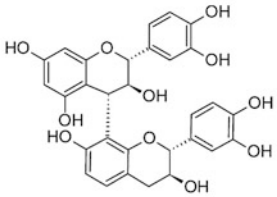
Flavonol
Quercetin: R = H
Isoquercitrin: R = *O*-β-D-glucosyl
Rutin: R = *O*-β-D-rutinosyl
Hyperoside: R = *O*-β-D-galactosyl
Quercetin-3-*O*-β-D-robinoside: R = *O*-robinosyl



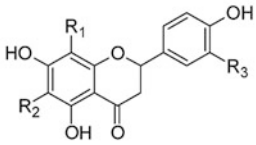
Flavone
Luteolin: $R_1 = R_3 = H$; $R_2 = R_4 = OH$
Luteolin-7-*O*-glucoside: $R_1 = R_3 = H$; R_2 = *C*-β-D-glucosyl; $R_4 = OH$
Chrysoeriol: $R_1 = R_3 = H$; $R_2 = OH$; $R_4 = OCH_3$
Orientin: R_1 = *C*-β-D-glucosyl; $R_2 = R_4 = OH$; $R_3 = H$
iso-orientin: $R_1 = H$; $R_2 = R_4 = OH$; R_3 = *C*-β-D-glucosyl
Vitexin: R_1 = *C*-β-D-glucosyl; $R_2 = OH$; $R_3 = R_4 = H$
iso-vitexin: $R_1 = R_4 = H$; $R_2 = OH$; R_3 = *C*-β-D-glucosyl



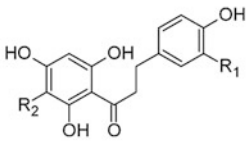
Monomeric flavanol
(+)- catechin



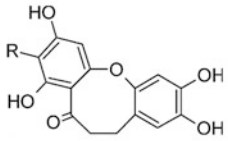
Oligomeric flavanol
Procyanidin B3



Flavanone
Dihydro-orientin: R_1 = *C*-β-D-glucosyl; $R_2 = H$; $R_3 = OH$
Dihydro-*iso*-orientin: $R_1 = H$; R_2 = *C*-β-D-glucosyl; $R_3 = OH$
Hemiphlorin: R_1 = *C*-β-D-glucosyl; $R_2 = R_3 = H$



Dihydrochalcone
Aspalathin: $R_1 = OH$; R_2 = *C*-β-D-glucosyl
Nothofagin: $R_1 = H$; R_2 = *C*-β-D-glucosyl



Cyclic dihydrochalcone
Aspalalinin: R = *C*-β-D-glucosyl

Fig. 181.2 Structures of main polyphenolic constituents in fermented rooibos (Adapted from Joubert et al. 2008a)

In vitro testing systems (where antioxidant structures and concentrations are well controlled) can evaluate the antioxidant properties of single/complex compounds and provide much needed data (i.e., possible mechanisms of action) to then be applied in more complex in vivo systems, but seldom represents effects occurring in vivo (Haenen et al. 2006). In vitro models do not take into account important factors such as metabolism, or the stability and activity of the putative metabolites and their bioavailability. A single assay (in vitro or in vivo) should not be applied to

determine the antioxidant activity of a compound or mixture of compounds and a multi-assay approach should always be taken based on the specific research question to be addressed (Griffiths et al. 2002; Collins 2005; Dilis and Trichopoulou 2010).

This chapter reviews information available on the antioxidant capacity of indigenous herbal tea rooibos (*Aspalathus linearis*) in vitro, in experimental animal models and in humans.

In Vitro Antioxidant Capacity

Rooibos is a rich and unique source of phytochemicals, including health beneficial polyphenols. An important property of rooibos is its of rooibos antioxidant capacity and free-radical scavenging ability similar to other polyphenolic compounds.

Total Phenolic Content

A well-known method for determining the total polyphenol content is the Folin–Ciocalteau technique, where the results are expressed as gallic acid equivalents (GAE) (Singleton and Rossi 1965). Improved methods for determining total phenolic content plants using this approach has recently been reviewed (Sánchez-Rangel et al. 2013).

The two most abundant dihydrochacones in green/unfermented rooibos tea, aspalathin and nothofagin, are degraded during fermentation with the concomitant formation of higher molecular weight products responsible for the known red color of traditional/fermented rooibos (Krafczyk et al. 2009b). To date, numerous studies have reported on the total polyphenol content of both the traditional/fermented and green/unfermented rooibos with Bramati et al. (2003) reporting a level of 68.4 mg GAE/g for green rooibos and 35.2 mg GAE/g for traditional rooibos prepared as a 1 % (w/v) aqueous extract with a steeping time of 10 min. In 2004, Joubert et al. reported on the total polyphenol and aspalathin content of different types of rooibos extracts. Aqueous extracts (100 g plant material in 1,000 mL boiling water steeped in a steam bath for 30 min), crude polymeric fractions, and ethyl acetate soluble fractions from both traditional and green rooibos were tested. The ethyl acetate fractions yielded the highest polyphenolic content of 675.2 mg GAE/g soluble solids and 558.2 mg GAE/g soluble solids for the green and traditional rooibos, respectively. Fermentation decreased the total polyphenol content of the traditional extracts (316–342 mg GAE/g soluble solids) when compared with the green rooibos extracts (393–399.2 mg GAE/g soluble solids).

A recent study reports a good correlation ($r = 0.99$) exists between the total polyphenol content and total antioxidant activity ($ABTS^{\cdot+}$ radical cation scavenging) of aqueous green rooibos extracts (Joubert et al. 2008a). Yoo et al. (2008) selected 17 common commercial herbs, including rooibos (presumably the traditional type), and compared their antioxidant capacities and phenolic contents under

Table 181.1 Total polyphenol, flavonol, flavanol content, and antioxidant capacity (ORAC) of a cup[a] of rooibos

Rooibos	Total polyphenol content (mg GAE)[b]	Total flavonols/ flavones (mg quercetin equivalents)[c]	Total flavanols/ proantho-cyanidins (mg catechin equivalents)[d]	ORAC (μmol TE)[e]
Traditional[f] rooibos	73.43 ± 1.79	33.44 ± 0.38	2.62 ± 0.02	1537.60 ± 27.4
Green[g] rooibos	106.46 ± 2.14	26.85 ± 0.28	6.02 ± 0.02	2093.57 ± 50.2

[a]One cup = 200 mL prepared by steeping one tea bag in 200 mL of freshly boiled water for 5 min
[b]Folin–Ciocalteu method
[c]360 nm method
[d]4-(Dimethylamino)-cinnamaldehyde (DMACA) method
[e]Oxygen radical absorbance capacity (ORAC) is expressed as μmol Trolox equivalents (TE) per cup using the fluorescein method. Values in columns are the means ± STD of ten samples done in triplicate
[f]Traditional = fermented
[g]Green = unfermented

the same conditions. A 70 % methanolic extract (repeated extraction) of each herb was prepared, and the Folin–Ciocalteu method was used to determine the total phenolic content. Rooibos contained 659.2 ± 2.1 mg GAE/100 g fresh herb total phenolics, of which 381.0 ± 1.6 mg catechin equivalents/100 g fresh herb were flavonoids (Yoo et al. 2008). Another study reported the total polyphenol content (Folin–Ciocalteau method) of an aqueous 1.5 % (w/v) rooibos infusion (5 min steeping) to be 881 ± 85.2 mg GAE/L infusion. Joubert et al. (2008b) prepared aqueous extracts of both traditional and green rooibos (10 % w/v with 5 min steeping) and reported 35.08 ± 1.69 mg GAE/100 g dried aqueous extract for green rooibos and 29.69 ± 1.49 mg GAE/100 g dried aqueous extract of traditional rooibos, again confirming decreases in total polyphenol content with fermentation. Other studies also report that the total polyphenol content of rooibos extracts decreases with fermentation (Standley et al. 2001; Marnewick et al. 2000). Recently, Marnewick et al.(2009a) reported on the total polyphenol content, flavonol and flavanol of a cup of rooibos (Table 181.1). The rooibos herbal teas used for these analyses were provided by Rooibos Ltd. (A Redelinghuys, Clanwilliam, South Africa).

The main phenolic component in rooibos, aspalathin, is decreased substantially (Figs. 181.3 and 181.4) after the fermentation process takes place, with less than 7 % remaining in the traditional/fermented rooibos (Joubert 1996). Bramati et al. (2003) reported that aspalathin, even after fermentation, still remains one of the major components of fermented rooibos. To date, large variations have been reported on the aspalathin and nothofagin content in rooibos samples, possibly due to genetic variations and differing fermetation processes (Joubert and Schulz 2006), as previously mentioned.

Van Heerden et al. (2003) reported that aspalathin found in the commercially cultivated rooibos plant (*Aspalathus linearis* (Burm.f.) Dahlg.) is absent in some of

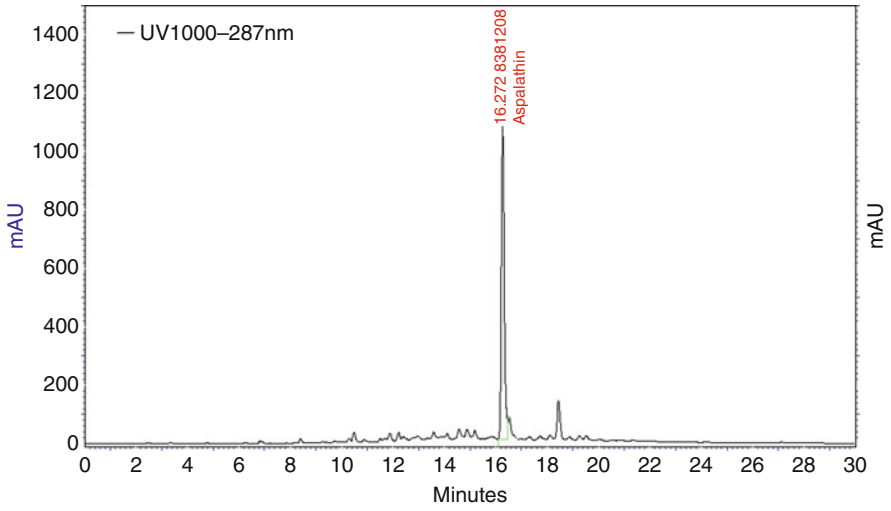


Fig. 181.3 HPLC chromatogram (287 nm) of a cup of green/unfermented rooibos showing the aspalathin (16.272 min) content (Unpublished data from the Oxidative Stress Research Centre, CPUT, South Africa)

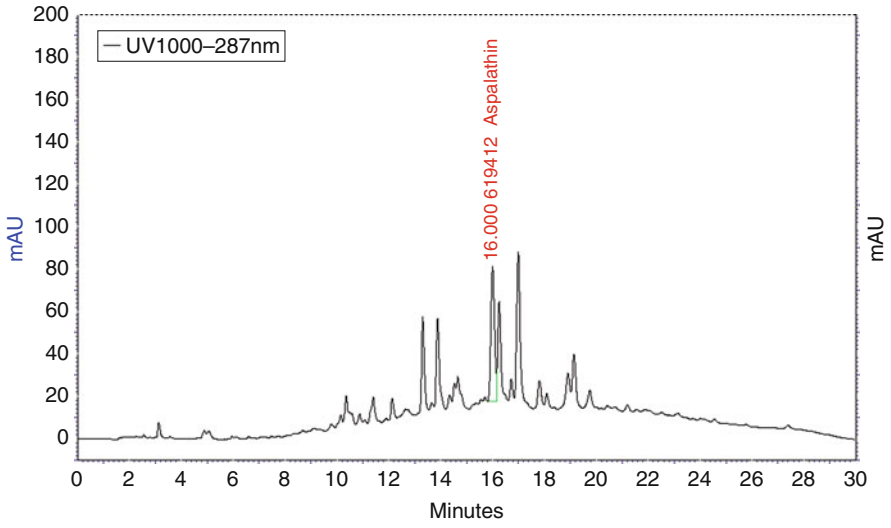


Fig. 181.4 HPLC chromatogram (287 nm) of a cup of traditional/fermented rooibos showing the aspalathin (16.272 min) content (Unpublished data from the Oxidative Stress Research Centre, CPUT, South Africa)

the wild rooibos populations, stressing the importance of quantitative and qualitative differences between wild and cultivated populations.

Several phenolic acids, i.e., caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, vanillic acid, and protocatechuic acid, are also been present in rooibos and add to the antioxidant capacity of the herbal tea (Rabe et al. 1994).

ABTS$^{\cdot+}$ (2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) Radical Cation) Scavenging Activity

The scavenging of ABTS^{+} is measured to assess the hydrogen-donating ability of flavonoids (also referred to as the trolox equivalent antioxidant capacity – TEAC) (Re et al. 1999; Arnao et al. 2001; Erel 2004).

Recently, Yoo et al. (2008) reported on the total antioxidant activity (using the ABTS radical scavenging capacity) of rooibos to be 631 ± 0.8 mg vitamin C equivalents per 100 g fresh herb. In another study by Almajano et al. (2008), using the TEAC assay, a hot water infusion (1.5 g plant material infused with 100 mL boiling water for 5 min) of presumable fermented/traditional rooibos was reported to be 746 ± 12.9 mmol trolox/L infusion. A study by Joubert et al. 2008c confirmed the antioxidant activity of both the fermented rooibos (1.72 ± 0.12 μmol trolox equivalents/mg aqueous extract) and green rooibos (2.37 ± 0.11 μmol trolox equivalents/mg aqueous extract) aqueous extracts (10 % w/v with 5 min steeping) using the ABTS^{+} assay. In 2003, Schulz et al. reported that the total antioxidant activity (measured as ABTS$^{\cdot+}$ scavenging ability) of green rooibos correlated ($r = 0.812$) with the aspalathin content of the plant material, but that its contribution to the total antioxidant capacity of traditional rooibos was negligible and that other polyphenolic compounds could have a contributory role.

The in vitro antioxidant activity of rooibos was confirmed in a study by Ivanova et al. (2005) when it was compared to that of Bulgarian medicinal plants using the TEAC assay to measure the ABTS^{+} radical scavenging ability. The rooibos extract, presumably traditional/fermented, was prepared by adding 200 mL boiling water to 1 g dried grinded plant material and steeped for 10 min.

Several studies have examined the antioxidant capacity of specific components of rooibos tea. A recent study by Snijman et al. (2009) reports that aspalathin and EGCG were the most potent scavengers of the ABTS$^{\cdot+}$ cation radical, followed by quercetin, then nothofagin, with iso-orientin, orientin, and luteolin being equally potent, but less than aspalathin but more than chrysoeriol. Hyperoside, rutin, and isoquercitrin were less potent than quercetin, their aglycone. Iso-vitexin and vitexin showed the lowest scavenging abilities. Krafczyk et al. (2009a) also reported a similar trend with a green rooibos tea extract (containing several antioxidants and 53 mg aspalathin per gram of freeze-dried extract) and aspalathin displaying the highest free-radical (ABTS^{+}) scavenging potency, compared with the other rooibos flavonoids. In this study, nothofagin also showed lower activity when compared to aspalathin.

Ferric Reducing Antioxidant Power (FRAP)

For the FRAP assay, antioxidants are evaluated as reductants of Fe^{3+} to Fe^{2+} (Benzie and Strain 1996). Joubert et al. (2008c) reported on the total antioxidant capacity of aqueous extracts of fermented and green rooibos using the FRAP assay. Green rooibos (1.98 ± 0.1 μmol TE/mg aqueous extract) yielded a higher ferric reducing ability than fermented rooibos (1.45 ± 0.08 μmol TE/mg aqueous extract). Results from the study showed a strong correlation between the total polyphenol content of the extracts and their antioxidant capacity.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

An early study by Von Gadow et al. (1997a) reported on the antioxidant activity of aqueous extracts (50 g dry leaves steeped in 1,000 mL boiling water for 10 min, as well as an additional 30 min) of traditional, green, and semi-fermented rooibos. Using the DPPH radical scavenging method, fermented rooibos produced a 83.4 % inhibition, with semi-fermented rooibos 81.9 % and green rooibos, the highest inhibition at 86.6 %. Another study by Von Gadow et al. (1997b) reported on the antioxidant activity of the major rooibos polyphenol, aspalathin, compared with that of other rooibos phenolic components. Results from this study showed aspalathin to inhibit the DPPH radical by 91.4 %. Yoo et al. (2008) reported a 78.5 % inhibition when using 100 μg/mL rooibos extract. Joubert et al. (2004) reported that a crude aspalathin fraction (461 mg/g soluble solids) was the best scavenger of the DPPH radical with 94.5 % inhibition, while the ethyl acetate fractions showed a 92.9 % and 91.8 % inhibition, the aqueous fractions 87.3 % and 83 % inhibition, and the crude polymeric fractions 87.7 % and 70.3 % inhibition for green and fermented rooibos, respectively (Joubert et al. 2004), again confirming the diminishing effect fermentation has on the total polyphenol content and antioxidant capacities of the various rooibos extracts. The study reported similar results for superoxide anion radical scavenging capacity. A twofold difference between the total antioxidant capacity of an aqueous extract (1 % w/v, steeping time 10 min) of green rooibos (0.8 Trolox meq/g) and fermented rooibos (0.4 Trolox meq/g) was also previously reported (Bramati et al. 2003). The level of aspalathin was almost 50 times higher in green rooibos than in the fermented rooibos, confirming that the processing of plant material decreased levels of the rooibos flavonoids, especially aspalathin (Bramati et al. 2003).

Peroxyl and Superoxide Anion Scavenging Ability

Yoshikawa et al. (1990) evaluated the antioxidant action and reactivity of a rooibos extract with various reactive oxygen species using electron spin resonance (ESR) spectrometry and reported that rooibos extracts scavenge superoxide as well as

hydroxyl radicals in vitro. Aqueous and 75 % ethanolic extracts of rooibos also showed good antioxidant activities, with the ethanol extract containing higher soluble phenolics and flavonoids than the water extract. Both extracts scavenged the hydroxyl radical, while the hydrogen-donating capacity and the scavenging activity of hydrogen peroxide were higher in the 75 % ethanol extracts (Lee and Jang 2004). Results from this study further showed aqueous and ethanolic rooibos extracts to protect DNA from peroxyl radical-induced damage by 79.0 % and 87.3 %, respectively. Although the results did not show protection against hydroxyl radicals, the authors concluded that rooibos flavonoids are responsible for several different antioxidant activities. Steenkamp et al. (2004) reported on the antioxidant scavenging potential of South African export herbal teas. Aqueous extracts of rooibos showed the best scavenging ability for both the superoxide anion and the hydroxyl radical while containing the highest flavonoid concentration when compared to the other export herbal teas.

For the ORAC (oxygen radical absorbance capacity) assays, antioxidants are evaluated as scavengers of 2,2′-azobis(2-amidino-propane)dihydrochloride (AAPH)-derived aqueous peroxyl radicals (Cao et al. 1993; Ou et al. 2001). The ORAC assay is a relatively new method and has not been extensively used to describe rooibos's antioxidant capacity. However, a recent study (Marnewick et al. 2011) reported on the ORAC value of cups of traditional and green rooibos teas (Table 181.1).

Rooibos Extracts as Antioxidants in Lipid Systems

Rooibos was traditionally prepared by brewing it on a stove for extended periods of heating. A study by Von Gadow et al. (1997c) reported that extending the brewing/steeping time (up to 25–30 min) of rooibos increased the antioxidant activity when using the Rancimat method, but no increase was observed with the β-carotene bleaching method.

Using a decoupled low-density lipoprotein (LDL) oxidation experiment, Krafczyk et al. (2009a) deduced that aspalathin's antioxidant properties seem to result from excellent radical scavenging and not metal chelating activity, while isoquercitrin showed a combination of strong metal chelating activity, thus protecting the LDL against induced oxidation, and a weaker radical scavenging ability.

The protective effect of ethanol/acetone soluble rooibos fractions (0.01 % w/v) against lipid peroxidation was also determined using rat liver microsomes in the presence of Fe^{2+} and absence of hydrogen peroxide (Marnewick et al. 2005). The green/unfermented rooibos extract exhibited the highest protective effect (91 %), while the fermented rooibos extract showed a 65 % protection (measured as decreased formation of thiobarbituric acid reactive substances – TBARS). It was reported that aspalathin and nothofagin comprised the major flavonoids in the green/unfermented rooibos fraction that was applied to mouse skin, while isoorientin, orientin, and aspalathin were the major flavonoids in the fermented rooibos fraction applied to the skin.

Joubert et al. (2008c) reported on the inhibition of Fe^{2+}-induced lipid peroxidation in rat liver microsomes. The green rooibos extract yielded a significantly higher protective effect (51.91 ± 1.57 % inhibition at 0.02 mg/mL aqueous extract) when compared to the fermented rooibos (41.07 ± 3.23 %).

It should also be noted that an in vitro prooxidant activity for rooibos was reported in 2005 (Joubert et al. 2005) using a linoleic acid emulsion peroxidation assay and oxidative degradation of deoxyribose in a Fenton system, dependent on the type of extract prepared, i.e., water extracts vs. enriched polyphenolic fractions. Pure aspalathin showed an in vitro prooxidant activity, while the prooxidant activity of the aqueous extracts and their crude polymeric fractions was linear to their flavonoid ($r = 0.971$, $P = 0.029$) and dihydrochalcone ($r = 0.977$, $P = 0.023$) content (Joubert et al. 2005). In the case of herbal medicines, it is thought that the prooxidant activity could be the cause of cytotoxicity and pro-apoptotic effects (Ueda et al. 2002).

Rooibos extracts have also been investigated as a food additive to protect against lipid peroxidation. In 2005, Hitomi et al. reported on the antioxidant activity of rooibos in lard and iron-enriched cookies, using the DPPH radical scavenging assay. The rooibos extract showed a strong dose-dependent antioxidant activity while showing similar activity in the lard preservation model. In the iron-enriched cookies, the rooibos extract inhibited lipid peroxidation, and the authors recommended rooibos as a useful additive to high-fat foods in order to prevent lipid peroxidation. This strategy could have important improved shelf-life applications, but should still be investigated.

Rooibos as Antioxidants in Various Cellular Systems

Yoshikawa et al. reported in the early 1990s that rooibos extracts (prepared in a 1:5 ratio with water, heating to 95 °C and letting it stand for a while) scavenged superoxide radicals without interfering or affecting the respiratory burst activity of human polymorphonuclear leukocytes, prepared from a healthy volunteer. Also in the early 1990s, Ito et al. (1991) reported the pretreatment (2 h prior to H_2O_2 treatment) of mouse leukemic cells (L5178Y) with aqueous extracts of rooibos leaves, to prevent H_2O_2-induced cell killing in a dose-dependent manner. It did not afford this protection when the rooibos extracts were added during the H_2O_2 treatment. The authors concluded that the protection could be because of secondary effects of the rooibos components increasing the cellular antioxidant activity during the preincubation and thus modifying the cells to be tolerable against induced oxidative stress.

Another study investigated the effect of rooibos on the growth and changes in growth parameters in cultured chick skeletal muscle cells to confirm correlation with its antioxidant properties (Lamošová et al. 1997). An aqueous extract of rooibos was prepared by adding 2,000 mL of boiling water to 3.5 g of dried leaves and stems and steeping for 20 min before filtration (0.22 μm membrane filter). Various dilutions of this extract were added to the cells. Results showed that the

rooibos extract inhibited the proliferation and growth of the muscle cells, and the authors concluded that this could be as a result of removal of reactive oxygen species needed for mitotic stimulation of cells, but it could not be excluded that other rooibos compounds might possess natural cytostatic properties.

Pretreating H_2O_2 exposed hamster lung fibroblasts (V79-4 cells) with a rooibos extract increased cell viability and protected gap-junction intercellular communication (related to anticancer mechanisms in multicellular organisms) (Yoo et al. 2008). The rooibos extract also induced two antioxidant enzymes', superoxide dismutase (SOD) and catalase (CAT) activities.

Very few studies to date have been done on the bioavailability and metabolism of specific rooibos phenolic constituents. Both major flavonoid constituents of green rooibos, aspalathin and nothofagin, show potent in vitro antioxidant capacity, with nothofagin being the less potent scavenger and weaker protector against Fe(II)-induced lipid peroxidation in a membrane assay system (Snijman et al. 2009; Krafczyk et al. 2009a). Recently, a study reported on the in vitro biotransformation of these two major rooibos constituents using Aroclor 1254-induced rat liver fractions. Two glucoronyl products and one sulfation product for aspalathin and only glucoronyl metabolites for nothofagin were detected as a result (Van der Merwe et al. 2010). The online HPLC $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ antioxidant assays showed that the putative glucoronidated aspalathin product lacked any radical scavenging properties. The nothofagin metabolite and sulfated aspalathin product were not tested, as it was established that these metabolites showed a low level of transformation and were non-active due to their structure. The authors concluded that there seems to be an important balance between the conjugated and nonconjugated forms of aspalathin, and it could affect the oxidative status in vivo (Van der Merwe et al. 2010). The bioavailability of rooibos is addressed in the "Human studies" section of this chapter.

Animal Studies

One of the very first studies to report on the antioxidant capacity of rooibos was that of Inanami et al. (1995). They examined the effects of chronic rooibos feeding on the accumulation of lipid peroxides in the brain, usually associated with aging. Using the TBARS assay, it was shown that rooibos significantly reduced the level of lipid peroxides in several brain regions (frontal cortex, occipital cortex, hippocampus, and cerebellum) of the older female Wistar rats (age 24 months), comparable to that of animals at the age of 5 weeks. The authors suggested that rooibos flavonoids functioned as free-radical scavengers in the brain and suppressed the formation of lipid peroxides.

Rooibos has also been shown to be a hepatoprotector via an antioxidative mechanism. Uličná et al. (2003) used a carbon tetrachloride (CCl_4)-induced rat model of liver injury. An aqueous extract of rooibos (5 g dried tea leaves and stems boiled for 10 min in 2,000 mL water and steeping for 20 min while cooling) was given to the rats 7 days before the CCl_4 administration, in addition to 5 mL/kg of the

rooibos given to the rats via gavage daily. The experiment lasted 10 weeks. Rooibos not only reversed steatosis and cirrhosis in the liver tissue but also significantly inhibited the increase of malondialdehyde (MDA), triacylglycerols, and cholesterol in the liver, with a similar effect on the plasma activities of aminotransferases (ALT, AST), alkaline phosphatase, and bilirubin concentrations. The authors concluded that the natural antioxidants and scavenging agents in rooibos might be effective herbal hepatoprotectors for patients with hepatopathies. A few years later, the effect of rooibos to support regeneration of rat liver after intoxication by CCl_4 (carbon tetrachloride) was investigated (Uličná et al. 2008). Results showed that rooibos significantly decreased the level of hepatic MDA in the early stage (7 days) of regeneration with both lower activities of ALT and AST and lower levels of total bilirubin. It was proposed that the rooibos antioxidant constituents scavenged the reactive oxygen species that increased due to the enhanced mitochondrial function that occurs during regeneration of the liver. The authors proposed rooibos as a coadjuvant for the therapy of liver diseases.

Using a similar study design, Kucharská et al. (2004) reported on the effect rooibos had on the antioxidant status of the liver using a rat CCl_4-induced liver damage model. Concentrations of reduced and oxidized coenzyme Q9 and α-tocopherol were measured with HPLC in liver tissue to determine the redox state and antioxidant capacity. Rats consuming rooibos showed increased antioxidant status in the livers, comparable with that of healthy animals, as shown by significantly reduced formation of MDA (lipid peroxidation marker), and improved levels of reduced coenzymes Q9 and α-tocopherol. Results from this study suggested that an adequate antioxidant status seemed to be important for protection, but that treatment with natural antioxidants may be better as indicated by the against liver damage improved coenzyme Q9 redox status.

Uličná et al. (2006) reported on the prevention of oxidative stress by rooibos in streptozotocin-induced diabetic rats. Aqueous and alkaline extracts of fermented rooibos were prepared and administered to the respective rats via daily gavage. Significantly decreased levels of MDA in the plasma, lens, and liver of these animals were reported. The levels of advanced glycation end products and advanced oxidation protein products, known to increase in the presence of oxidative stress, were also significantly lowered by the rooibos extracts. The authors recommended rooibos as a preventative measure against microvascular and/or macrovascular diabetic complications, largely due to rooibos being an effective antioxidant in both hydrophilic and hydrophobic biological systems.

Marnewick et al. (2003) reported on the effect chronic feeding of aqueous extracts of both fermented and green rooibos had on the modulation of oxidative stress in male Fischer rats. Rooibos leaves and stems (both fermented and green) were prepared by adding 100 mL freshly boiled tap water to 2 g of plant material, while steeping for 30 min before filtering, cooling, and feeding it to the rats in their water bottles for 10 weeks. Results from this study showed that although fermented and green rooibos extracts did not have any effect on the antioxidant capacity (measured as ORAC) of the liver, both extracts significantly improved the glutathione (GSH) redox status of the liver by reducing the level of oxidized

glutathione (GSSG) and thus increasing the GSH:GSSG ratio significantly. In the presence of reactive species, GSH is rapidly oxidized to GSSG, and in its turn, exported from cells. Intracellular levels of GSH, GSSG, or the ratio of GSH: GSSG serve as indicators of oxidative stress (Fang et al. 2002). Simultaneously, the rooibos extracts also significantly enhanced the activities of the important hepatic phase II drug-metabolizing enzymes, glutathione S-transferase alpha and UDP-glucoronosyl transferase. These results suggest that rooibos represents that rooibos widely consumed a promising chemopreventive tool for humans as this herbal tea is consumed.

Another application where the antioxidant properties of rooibos may play an important role is in chemoprevention. Reactive oxygen species play an important role in carcinogenesis as they regulate critical cell proliferation and apoptosis events (Klaunig and Kamendulis 2004). A number of experimental animal studies have reported on the cancer-modulating properties of rooibos. Recently, Marnewick et al. (2009b) showed rooibos extracts to protect against fumonisin B_1-induced cancer promotion in male Fischer rats. Fermented rooibos (prepared as an aqueous extract, 2 % w/v) significantly decreased the FB_1-induced lipid peroxidation (TBARS assay) in the liver. Both fermented and green rooibos extracts reduced the relative number of larger $GSTP^+$ liver foci (precancerous lesions), while green rooibos also significantly reduced the total number of foci. The authors concluded that part of the reason rooibos afforded the protection against cancer promotion could be as a result of the modulation of the FB_1-induced oxidative stress by rooibos, but other possible mechanisms could also be involved and needed further investigation.

Also recently, Sissing et al. (2011) reported on the modulation of methylbenzylnitrosamine (MBN)-induced esophageal squamous cell carcinogenesis with green rooibos (prepared as a 2 % w/v aqueous extract and administered to male Fischer rats for 25 weeks after MBN initiation) reducing the mean total papilloma size by 87 %. This reduction correlated ($r = 0.99$; $P < 0.002$) with the daily intake of total polyphenols and flavonol/xanthones by the animals. Results also confirmed that fermentation resulted in reduced polyphenolic constituents with a resultant reduced inhibition of papilloma development. The authors concluded that due to many confounding factors such as lifestyle and bioavailability, the role of tea polyphenols in human cancer prevention remain unsure.

Previous studies have also reported on the topical application of rooibos extracts to modulate tumor promotion in mouse skin. Using a two-stage mouse skin carcinogenesis model, Marnewick et al. (2005) reported ethanol/acetone soluble fractions prepared from methanolic extracts of fermented and green rooibos inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion. The fermented rooibos extract afforded a 75 % inhibition, while the green rooibos extract a 60 % inhibition, in terms of a decreased tumor volume, mean number of tumors, and delayed tumor development. In this topically applied model, it seemed that the flavonol/flavone content played a secondary role to the flavanol/proanthocyanidin content, as the fermented extract afforded the better protection, in contrast to findings in the other carcinogenesis studies. More recently, using ethanol/acetone soluble fractions prepared from ethanolic extracts of fermented and green rooibos,

Petrova et al. (2011) reported on the protective effect against ultraviolet B (UVB)-promoted skin tumorigenesis in SKH-1 mice, using 7,12-dimethylbenz[a] anthracene (DMBA) as an initiator. The results from this study confirmed the results from Marnewick et al. (2005), in that fermented rooibos afforded the best protection with a 91 % reduction in the number of tumors that developed per mouse, followed by green rooibos with a reduction of 75 %.

Anti-inflammatory effects of rooibos have also been reported in a rat colitis model (dextran sodium sulfate (DSS)-induced colitis), where rats consuming rooibos (1.6 g unfermented rooibos leaves steeped in 100 mL 92 °C water for 15 min with separation of the insoluble residue) for 4 weeks had significantly increased levels of serum SOD, while urine 8-hydroxy-2′-deoxyguanosine levels were significantly decreased (Baba et al. 2009). After the DSS treatment, the levels of SOD remained higher in the rooibos group and prevented a decrease in hemoglobin level, as seen in the control group. No significant differences in clinical symptoms were shown between the rooibos-consuming animals and the control animals. The authors concluded that rooibos (in this instance, green/unfermented rooibos) reduced oxidative DNA damage via its in vivo antioxidative activity and proposed rooibos flavonoids to play a role in the increased SOD levels. Further studies are still needed to confirm the use of rooibos in the treatment of inflammatory bowel diseases. As a result of the outcome of the study, it was suggested that the routine intake of rooibos may be safe in modulating oxidative stress in children (Baba et al. 2009).

Pantsi et al. (2011) reported on the cardiac protective properties of rooibos. In this study, male Wistar rats consumed fermented and green rooibos (2 g leaves per 100 mL freshly boiled water, steeped for 30 min) for 7 weeks as the sole source of drinking fluid. At the end of the feeding period, the hearts were excised and mounted on a working heart perfusion apparatus. Aortic output recovery was measured as a functional parameter, as well as various biochemical parameters in the heart including the redox status of glutathione and various cell-signaling parameters. Results showed that both green and fermented rooibos significantly improved the aortic output recovery of hearts by 61.58 % and 61.06 %, respectively, but with no effect on the redox status of GSH. Both green and fermented rooibos also significantly decreased poly (ADP-ribose) polymerase (PARP) cleavage, and the authors suggested that protection afforded by rooibos could be as a result of the inhibition of apoptosis. The fermented rooibos also showed a significant decrease in cleaved caspase-3, another apoptosis marker, supporting the case that rooibos flavonols have an important anti-apoptotic role to play. Future studies were recommended to determine the vascular effects of rooibos compounds and/or their respective metabolites to confirm its potential as a therapeutic substance.

Other biological activities that have been described for rooibos also include the modulation of metabolic abnormalities/disturbances, with rooibos protecting the liver against lipid storage in hyperlipidemic mice with an increased energy intake via a Western-type diet (Beltrán-Debón et al. 2011). Kawano et al. (2009) also reported on the effect of aspalathin, the unique rooibos flavonoid, on glucose

metabolism. Using a type 2 diabetes mouse model, results from the study showed dietary aspalathin to suppress the increase in fasting glucose levels in male db/db mice.

Human Studies

To consider the question whether rooibos flavonoids are physiologically relevant antioxidants, thought has to be given to their bioavailability. Courts and Williamson (2009) investigated the absorption of methylated and glucuronidated aspalathin, a *C*-glycosyl flavonoid from rooibos, in humans. Six healthy individuals consumed 300 mL of an aspalathin-rich green rooibos tea solution (14 g leaves added to 1 L 100 °C water, extracted 3 times for 10 min). The 300 mL green rooibos contained 91.2 mg aspalathin. The participants also consumed a standard polyphenol-free meal at three occasions (1, 5, and 9 h) after taking the rooibos. The duration of the study was 24 h, with urine samples collected every 2 h. Results from this study reported the presence of unhydrolyzed methylated metabolites of aspalathin in the urine, revealing that deglycosylation is not needed for *C*-glycosyl flavonoid absorption in humans. Previously, results from a long-term feeding experiment in pigs also showed intact aspalathin metabolites in the urine with very limited absorption taking place (0.16–0.87 %) after 7 days of treatment (Kreuz et al. 2008).

Stalmach et al. (2009) conducted an investigation into the bioavailability of rooibos flavonoids using ten volunteers, each whom consumed 500 mL of a "ready-to-drink" fermented or green rooibos beverage after fasting, collecting plasma and urine samples before, and at 0.5, 1, 2, and 5 h after consuming the rooibos beverage, while urine was also collected up to 24 h after consumption. Results from HPLC–PDA–MS analyses again confirmed the poor bioavailability of the dihydrochalcone and flavanone *C*-glucosides in the fermented and green rooibos beverages as, only trace amounts of metabolites were shown in the urine after 24 h with no detectable quantities in the plasma and showed a rapid turnover and removal of the metabolites from the circulatory system. As most of the aspalathin metabolites were excreted within 5 h of consumption and the urinary excretion of the eriodictyol-*O*-sulfate within 5–12 h, the authors suggested passage from small to large intestine with further action by the colonic microflora producing low molecular weight phenolic acids.

In a recent randomized crossover control trial, 12 healthy males consumed water, green rooibos, or an isolated fraction of green rooibos (containing 0.3 g/0.5 g aspalathin) (Breiter et al. 2011). Standardized meals, with a low flavonoid and vitamin C and E content, were consumed 30 min, 2, and 6 h after consuming the respective rooibos treatments. Blood and urine samples were collected before and at various times following the treatments. Results showed that the main urinary metabolite was methylated aspalathin, with six other metabolites of aspalathin and nothofagin also identified. The plasma showed trace quantities of unchanged green rooibos flavonoids. The peak concentration detected in the plasma was 0.76 nmol (0.26 % of total flavonoid intake) and 0.41 nmol after the intake of

the isolated green rooibos fraction. No increase in the plasma antioxidant capacity (measured by ORAC) could be shown, but rather a significant decrease, correlating with the poor recovery rates of the flavonoids. This decrease in antioxidant capacity had also been reported by Sauter in 2004. The authors could not exclude that the consumption of carbohydrates as an energy source could be associated with this loss in antioxidant capacity in the blood, a phenomenon previously shown by Prior et al. (2007). Breiter et al. (2011) have shown limited bioavailability of the dihydrochalcone-, flavone-*C*-, and flavonol-*O*-glycosides in green rooibos and the isolated fraction, and suggested that further studies be conducted using other parameters, i.e., malondialdehyde, glutathione to assess the in vivo antioxidant efficacy of rooibos flavonoids. Table 181.2 serves as a summary of the published human studies (in chronological order) involving rooibos.

Two other human studies have reported on the use of other biomarkers for rooibos. Nikolova et al. (2007) reported on the antioxidative effects of rooibos on workers occupationally exposed to lead. 75 male workers consumed either rooibos or a placebo, whereafter several biochemical parameters were measured in the blood. Consuming rooibos increased the levels of reduced GSH levels by 47.8 % and also significantly decreased plasma MDA levels, hereby positively modulating the antioxidant indices. Another pioneering study to report on the in vivo modulating effect of rooibos is that of Marnewick et al. (2011). This study investigated the effects of fermented rooibos on oxidative stress in adults at risk for cardiovascular disease. Forty participants (26 females, 14 males) consumed six cups (one cup constitutes one tea bag steeped in 200 mL freshly boiled water for 5 min) of traditional/fermented rooibos per day for 6 weeks followed by a crossover control period when water was consumed. Similarly, results from this study also failed to show any increase in the plasma antioxidant capacity (measured by ORAC), advising that ORAC should not be used as the sole marker for determining the in vivo antioxidative capacity of rooibos. Fasting blood samples from this study did show a positive modulation of the lipid profile of the participants with the consumption of fermented rooibos resulting in a significant decrease in serum LDL cholesterol and triacylglycerols while also significantly increasing the HDL-cholesterol levels. In addition, consuming six cups of rooibos daily also improved the redox status of the participants as shown by the significant reduction in lipid peroxidation (measured as conjugated dienes and TBARS) as well as the significant increase in GSH levels, with a resultant increase in the GSH: GSSG ratio; such changes in redox markers are relevant to heart disease. Wanjiku (2009) also reported no significant change in the antioxidant capacity (measured as ORAC and FRAP) of healthy volunteers that consumed an acute dose of 500 mL traditional/fermented rooibos at 45, 90, and 180 min after consumption.

Interestingly, Persson et al. (2010) reported the cardiovascular effects of rooibos prior to the study by Marnewick et al. (2011). This study investigated the effects of rooibos (and other teas) on the angiotensin-converting enzyme (ACE) and nitric oxide (NO) in 17 healthy volunteers as a result of previous reports suggesting tea to reduce cardiovascular mortality. The volunteers (non-fasting) consumed 400 mL rooibos infusion (10 g leaves in 400 mL freshly boiled water, steeping for 10 min),

Table 181.2 Summary of human studies reporting on various biological activities of rooibos

Biological activity	Subjects	Dose	Outcome	References
Iron absorption	Ten adult males	Single dose 200 mL fermented rooibos tea with milk and sugar	No effect on iron status, similar to water	Hesseling et al. (1979)
Antiallergic properties	Seven adults diagnosed with asthma and hay fever	Three doses of 500 mL fermented rooibos tea (25 g tea leaves per 1 L boiling water, steeping for 5 min). A rooibos poultice was also applied to the forearms 15 min before skin–prick test	No antihistaminic effects	Hesseling and Joubert (1982)
Dermatological and antiviral effects	Patients diagnosed with viral infection (*herpes simplex*) and itching	Rooibos infusion once per week	Decreased incidence of *herpes simplex* within 2–3 days. Decreased itching in patients with atopic dermatitis	Skindo and Kato (1991)
Antioxidant status	Twenty subjects	Two tablets (250 mg) taken daily for 2 weeks. Tablets contained an aspalathin-rich extract prepared from green rooibos	No changes in the antioxidant status ($ABTS^{\cdot+}$). No changes in Cu^{2+}-induced LDL oxidation	Sauter (2004)
Iron absorption	One hundred and seventy-five school children	Two cups (200 mL per cup) of fermented rooibos with milk and sugar for 16 weeks	No adverse effects on iron status of children	Breet et al. (2005)
Oxidative stress status	Seventy-five male lead factory workers	Fermented rooibos taken for 8 weeks	Significant decreased lipid peroxidation (plasma MDA levels). Significant increased (47.8 %) plasma GSH levels	Nikolova et al. (2007)
Antioxidant status	Eight healthy males	Single oral dose of 500 mL fermented rooibos with blood taken at 0 h, 45 min, 90 min, 180 min	No significant changes in antioxidant parameters (ORAC, FRAP, TP, tGSH, GSH: GSSG)	Wanjiku (2009)

Antiwrinkle activity	Twenty health women	Commercial mixture of *C. sinensis* and *A. linearis* applied twice daily to the inner forearm for 28 days	Significant reduction in wrinkles (9.9 %) with no improved skin moisturizing action	Chuarienthong et al. (2010)
Postprandial oxidative stress and lipid profile modulation	Fourteen healthy volunteers (seven male, seven female)	Single oral dose of 500 mL fermented rooibos with sugar taken after a standardized fat meal. Blood taken at 0 h, 2 h, 4 h, 6 h	Significant reduction in plasma glucose (6 h), insulin (4 h), total cholesterol (2, 4, 6 h), LDL (2, 4, 6 h), triglycerides (4, 6 h) and hs-CRP (6 h) levels. Significant reduction in lipid peroxidation (CDs, TBARS) at 2, 4 h with significant increase in tGSH levels (2 h). Also showed a significant increase (4 h) in plasma antioxidant activity (TEAC)	Francisco (2010)
Cardiovascular effects	Seventeen healthy volunteers (nine males, eight females)	Single oral dose of 400 mL fermented rooibos (10 g leaves per 400 mL boiling water, 10 min infusion). Blood taken at 0 h, 30 min, 60 min, and 3 h	Significant inhibition of ACE activity at 30 and 60 min, specifically for the ACE II genotype. No effect was shown on the levels of nitric oxide	Persson et al. (2010)
Hepatotoxicity	Single case study with 42-year-old woman diagnosed with low-grade B-cell malignancy, Waldenström macroglobulinemia	One liter fermented rooibos daily for ± 2 weeks (1 tsp leaves per cup boiling water, steeping for 10 min)	Elevated plasma levels of ALT, GGT, ALP with other chemical pathology markers within normal range. No reexposure was done to confirm rooibos as the cause	Sinisalo et al. (2010)
Hydration	Twenty-three healthy male collegiate wrestlers	Fermented rooibos infusion (Reddrox) taken by the athletes after a 2 h wrestling practice (dehydration). Measure various parameters after 1 h of rehydration	Rooibos effect comparable to that of water in promoting rehydration	Utter et al. (2010)
Antioxidant status	Fifteen healthy volunteers	Single oral dose of 500 mL fermented rooibos, green rooibos (1.5 g rooibos extract per L) or	Significant increase in the plasma antioxidant capacity (TRAP) with a 6.6 % increase at 1 h for fermented	Villaño et al. (2010)

(*continued*)

Table 181.2 (continued)

Biological activity	Subjects	Dose	Outcome	References
		water. Blood was taken at 0 h, 30 min, 1, 2, and 5 h	rooibos and 2.9 % increase for green rooibos	
Antioxidant status	Twelve healthy male volunteers	Single oral dose of 500 mL green rooibos (10 g leaves per 500 mL boiling water, steeping time of 10 min), aspalathin-rich fraction or water was taken	No significant increase in serum antioxidant capacity (ORAC) up to 8 h after ingestion	Breiter et al. (2011)
Hair growth	Sixty-nine males with varying degrees of androgenetic alopecia	Optimized botanical blend also containing green rooibos was topically applied for 12 weeks	Significant increased hair density and number of anagen follicles. Significant increased growth rate of hair	Glynn (2010)
Modulation of oxidative stress, lipid profile modulation	Forty adults (14 males and 26 females) at risk for cardiovascular disease	Six cups of fermented rooibos (one tea bag per 200 mL boiling water, steeping for 5 min) daily for 6 weeks	Significant decrease in lipid peroxidation (CDs, TBARS). Significant increase in tGSH; GSH: GSSG ratio. Positive modulation of the lipid profile (significant decreased LDL, triacylglycerols, increased HDL)	Marnewick et al. (2011)

$ABTS^{\cdot+}$ 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) radical cation, *ACE* angiotensin-converting enzyme, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CD* conjugated dienes, *tGSH* total glutathione, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *hs-CRP* high-sensitive C-reactive proteins, *NO* nitric oxide, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *ORAC* oxygen radical absorbance capacity, *TRAP* total radical-trapping antioxidant potential assay, *FRAP* ferric reducing antioxidant potential, *TBARS* thiobarbituric acid reactive substances

with venous blood collected before and at various time periods (30 min, 60 min, and 3 h) after the consumption. Results showed rooibos to significantly inhibit ACE activity after 30 and 60 min, and specifically genotype II at 60 min. No significant changes were shown for NO levels, blood pressure or heart rate at the various time points. The authors pointed out that previously protective effects displayed by the rooibos could not only be ascribed to the antioxidant action, but that ACE inhibition should be added as another mechanism, specifically where cardiovascular disease is concerned. More recently, Persson (2011) reported that rooibos inhibited ACE activity using a "mixed inhibitor" mechanism, as shown by the results of V_{max} and K_m.

In 2010, Villaño et al. reported green and fermented rooibos to increase the plasma antioxidant capacity in healthy humans. In this acute study, 15 healthy volunteers consumed 500 mL water or "ready-to-drink" rooibos beverages (1.5 g/L rooibos extract powder) with blood collection taking place before and at 30 min, 1, 2, and 5 h after consumption. Consumption of the fermented rooibos beverages resulted in a 4.8 % increase in the "chain-breaking" total antioxidant capacity (measured by TRAP assay) after 30 min, while peaking after 1 h at 6.6 % increase ($P < 0.05$) and declining to 4.9 % ($P < 0.05$) at 2 h. The green rooibos beverage only caused an increase of 2.7 % after 2 h. Both rooibos beverages increased glycemia after 30 min of being ingested with no changes in plasma uric acid or the total cholesterol and triacylglycerol levels. This increase in plasma antioxidant capacity after an acute dose of rooibos is the first report to date, but current bioavailability studies could not be used to explain this. The authors suggested a rapid removal of rooibos metabolites from the circulatory system, and concluded that rooibos could be a useful source of dietary antioxidants.

Francisco (2010) investigated the modulation of postprandial oxidative stress by rooibos in normolipidemic volunteers. In a crossover control study, 14 participants consumed 500 mL of a fermented rooibos beverage (2 % w/v prepared with freshly boiled water with a steeping time of 5 min), sweetened with sucrose. The rooibos beverage was taken 15 min after the participants consumed a standardized fat meal with blood samples taken at 0 h, 2, 4, and 6 h post ingestion. A commercial soda beverage was used as the control beverage. The plasma glucose and insulin levels were significantly decreased at the 6 h and 4 h time interval, respectively, when compared to the control, while significantly decreased total cholesterol, LDL, and triacylglycerols were also shown at various time intervals. The inflammatory biomarker, hs-CRP was also shown to be significantly decreased at 6 h time interval in participants that consumed the rooibos beverage. The plasma antioxidant capacity (measured as TEAC) also increased significantly at the 4 h time interval. A significant decreased lipid peroxidation level (measured as conjugated dienes and TBARS) were also shown at time intervals 2 and 4 h after consuming the rooibos beverage. Important factors to take into account when comparing the results from human studies include both the timing of when the blood was taken and whether the participants fasted, as some studies had reported blood taking to take place after an overnight fast, while others had reported 30 min–1 h after rooibos consumption, with or without food intake.

Conclusions

The nature of in vivo studies makes it very problematic to deduce specific antioxidant mechanisms involved in cellular protective processes. In vitro assays are simpler and serve as good screening systems for antioxidant activity, while providing important information on the possible mode of action (Haenen et al. 2006). Both systems are necessary when determining possible antioxidant capacity and health-promoting properties. In general, it seems that phytochemical compounds, especially the polyphenols, are accepted to be responsible for the various health-promoting properties being reported, but that further evidence from well-planned human intervention studies are needed to confirm this.

Data from in vitro studies have shown rooibos polyphenols and rooibos extracts to possess antioxidant capacity, while data from the few human studies to date are looking promising. In spite of rooibos flavonoids' low bioavailability, an increasing number of papers, as discussed in this chapter, are reporting on very promising in vivo activities derived from human studies, i.e., positive modulation of the lipid profile, improved GSH redox status, decreased lipid peroxidation, and increased antioxidant capacities. These encouraging results definitely warrant further studies to elucidate the possible health-promoting properties of rooibos.

References

Almajano MP, Carbó R, Jiménez JAL, Gordon MH (2008) Antioxidant and antimicrobial activities of tea infusions. Food Chem 108:55–63

Arnao MB, Cano A, Acosta M (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 73:239–244

Baba H, Ohtsuka Y, Haruna H, Lee T, Nagata S, Maeda M, Yamashiro Y, Shimizu T (2009) Studies of anti-inflammatory effect of rooibos tea in rats. Pediatr Int 51:700–704

Beecher GR (2003) Overview of dietary flavonoids: nomenclature, occurrence and intake. J Nutr 233:3248S–3254S

Beltrán-Debón R, Rull A, Rodrígues-Sanabria F, Iswaldi I, Herranz-López M, Aragonès G, Camps J, Alonso-Villaverde C, Menéndez JA, Micol V, Segura-Carretero A, Joven J (2011) Continuous administration of polyphenols from aqueous rooibos (*Aspalathus linearis*) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice. Phytomedicine 18:414–424

Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76

Blommeart KLJ, Steenkamp J (1978) Tannien- en moontlike kafeieninhoud van rooibos tee, *Aspalathus* (subgen. Nortiera) *linearis* (Burm. Fil) R Dalhlgr. Agroplantae 10:49

Bramati L, Aquilano F, Peitta P (2003) Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC-UV and determination of the total antioxidant capacity. J Agric Food Chem 51:7472–7474

Breet P, Kruger HS, Jerling JC, Oosthuizen W (2005) Actions of black tea and rooibos on iron status of primary school children. Nutr Res 25:983–994

Breiter T, Laue C, Kressel G, Gröll EUH, Hahn A (2011) Bioavailability and antioxidant potential of rooibos flavonoids in humans following the consumption of different rooibos formulations. Food Chem 128:338–347

Cao G, Alessio HM, Cutler RG (1993) Oxygen-radical absorbance capacity assay for antioxidants. Free Radical Biol Med 14:303–311

Chuarienthong P, Lourith N, Leelapornpisid P (2010) Clinical efficacy comparison of anti-wrinkle cosmetics containing herbal flavonoids. Int J Cosmetic Sci 32:99–106

Collins A (2005) Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols. Am J Clin Nutr 81:261S–267S

Courts FL, Williamson G (2009) The *C*-glycoside flavonoid, aspalathin, is absorbed, methylated and glucuronidated intact in humans. Mol Nutr Food Res 53:1104–1111

Dilis V, Trichopoulou A (2010) Assessment of antioxidants in foods and biological samples: a short critique. Int J Food Sci Nutr 61(5):441–448

Erel O (2004) A novel automated direct measurement method of total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 37(4):277–285

Fang Y-Z, Yang S, Wu G (2002) Free radicals antioxidants and nutrition. Nutr 18:872–879

Francisco NM (2010) Modulation of postprandial oxidative stress by rooibos (*Aspalathus linearis*) in normolipidemic individuals. M.Tech. (Biomedical Technology) Thesis, Cape Peninsula University of Technology, Bellville

Glynn KM (2010) From in vitro to in vivo: corporate development and efficacy of a topical hair growth agent derived from natural extracts. MSc (Biology) Thesis, Grand Valley State University, Michigan

Griffiths HR, Moller L, Bartosz G, Bast A, Bertoni-Freddari C, Collins A, Cooke M, Coolen S, Haenen G, Hoberg A-M, Loft S, Lunec J, Olinski R, Parry J, Pompella A, Poulsen H, Verhagen H, Astley SB (2002) Biomarkers. Mol Aspects Med 23:101–208

Haenen GRMM, Arts MJTJ, Bast A, Coleman MD (2006) Structure and activity in assessing antioxidant activity in vitro and in vivo: a critical appraisal illustrated with the flavonoids. Environ Toxicol Pharm 21:191–198

Hesseling PB, Klopper JF, Van Heerden PDR (1979) The effect of rooibos tea on iron absorption [Afrikaans]. S Afr Med J 55:631–632

Hesseling PB, Joubert JR (1982) The effect of rooibos tea on the type I allergic reaction. S Afr Med J 62:1037–1038

Hitomi ERI, Miura Y, Mihara K, Onishi S, Hara N, Nakano M (2005) The antioxidative activity of rooibos tea in iron-enriched cookies. J Jpn Soc Food Sci 52:594–598

Inanami O, Asanuma T, Inukai N, Jin T, Shimokawa S, Kasai N, Nakano M, Sato F, Kuwabara M (1995) The suppression of age-related accumulation of lipid peroxides in rat brain by administration of rooibos tea (*Aspalathus linearis*). Neurosci Lett 196:85–88

Ito A, Shinohara K, Kator K (1991) Protective action of rooibos tea (*Aspalathus linearis*) extract against inactivation of L5178Y cells by H_2O_2. In: Proceedings of the international symposium on tea science, The Organizing Committee of ISTS, Shizuoka, pp 381–384

Ivanova D, Gerova D, Chervenkov T, Yankova T (2005) Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacol 96:145–150

Joubert E (1996) HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. Food Chem 55:403–411

Joubert E, Winterton P, Britz TJ, Ferreira D (2004) Superoxide anion and α, α-diphenyl-β-picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. Food Res Int 37:133–138

Joubert E, Winterton P, Britz TJ, Gelderblom WCA (2005) Antioxidant and pro-oxidant activities of aqueous extracts and crude polyphenolic fractions of rooibos (*Aspalathus linearis*). J Agric Food Chem 53:10260–10267

Joubert E, Schulz H (2006) Production and quality aspects of rooibos tea and quality products. A review. J Appl Bot Food Qual 80:138–144

Joubert E, Gelderblom WCA, Louw A, De Beer D (2008a) South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylicoides* – a review. J Ethnopharmacol 119:376–412

Joubert E, Manley M, Botha M (2008b) Evaluation of spectrophotometric methods for screening of green rooibos (*Aspalathus linearis*) and green honeybush (*Cyclopia genistoides*) extracts for high levels of bio-active compounds. Phytochem Anal 19:169–178

Joubert E, Richards ES, Van der Merwe JD, De Beer D, Manley M, Gelderblom WCA (2008c) Effect of species variation and processing on phenolic composition and in vitro antioxidant activity of aqueous extracts of Cyclopia spp. (Honeybush tea). J Agric Food Chem 56:954–963

Kawano A, Nakamura H, Hata S, Minakawa M, Miura Y, Yagasaki K (2009) Hypoglycaemic effect of aspalathin, a rooibos tea component from *Aspalathus linearis*, in type 2 diabetic model db/db mice. Phytomedicine 16:437–443

Klaunig JE, Kamendulis LM (2004) The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol 44:239–267

Krafczyk N, Woyand F, Glomb MA (2009a) a structure-antioxidant relationship of flavonoids from fermented rooibos. Mol Nutr Food Res 53:635–642

Krafczyk N, Heinrich T, Porzel A, Glomb MA (2009b) Oxidation of the dihydrochalcone aspalathin leads to dimerization. J Agric Food Chem 57:6838–6843

Kreuz S, Joubert E, Waldmann K-H, Ternes W (2008) Aspalathin, a flavonoid in *Aspalathus linearis* (rooibos), is absorbed by pig intestine as a *C*-glycoside. Nutr Res 28:690–701

Kucharská J, Uličná O, Gvozdjáková A, Sumbalová Z, Vančová O, Božek P, Nakano M, Greksák M (2004) Regeneration of coenzyme Q9 redox state and inhibition of oxidative stress by rooibos tea (*Aspalathus linearis*) administration in carbon tetrachloride liver damage. Physiol Res 53:515–521

Lamošová D, Juráni M, Greksák M, Nakano M, Vaneková M (1997) Effect of rooibos tea (*Aspalathus linearis*) on chick skeletal muscle cell growth in culture. Comp Biochem Physiol 16C:39–45

Lee E-J, Jang H-D (2004) antioxidant activity and protective effect on DNA strand scission of rooibos tea (*Aspalathus linearis*). BioFactors 21:285–292

Louwrens H, Rautenbach F, Venter I (2009) South African dietary total antioxidant capacity based on secondary intake data in relation to dietary recommendations. S Afr J Clin Nutr 22(4):195–202

Marnewick JL, Gelderblom WCA, Joubert E (2000) An investigation on the antimutagenic properties of South African herbal teas. Mutat Res 471:157–166

Marnewick JL, Joubert E, Swart P, Van Der Westhuizen F, Gelderblom WCA (2003) Modulation of hepatic drug metabolizing enzymes and oxidative status by green and black (*Camellia sinensis*), rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*) teas in rats. J Agric Food Chem 51:8113–8119

Marnewick JL, Joubert E, Joseph S, Swanevelder S, Swart P, Gelderblom WCA (2005) Inhibition of tumour promotion in mouse skin by extracts of rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*), unique South African herbal teas. Cancer Lett 224:193–202

Marnewick JL (2009a) Rooibos and honeybush: recent advances in chemistry, biological activity and pharmacognosy. In: Juliana HR, Simon JE, Ho C-T (eds) African natural plant products: new discoveries and challenges in chemistry and quality, ACS symposium series 1021. Oxford University Press, Oxford, pp 277–294

Marnewick JL, Van der Westhuizen FH, Joubert E, Swanevelder S, Swart P, Gelderblom WCA (2009b) Chemoprotective properties of rooibos (*Aspalathus linearis*), honeybush (*Cyclopia intermedia*) herbal and green and black (*Camellia sinensis*) teas against cancer promotion induced by fumonisin B_1 in rat liver. Food Chem Toxicol 47:220–229

Marnewick JL, Rautenbach F, Venter I, Neethling H, Blackhurst DM, Wolmarans P, Macharia M (2011) Effects of rooibos (*Aspalathus linearis*) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. J Ethnopharmacol 133:46–52

McKay DL, Blumberg J (2007) A review of the bioactivity of South African herbal teas: rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia intermedia*). Phytother Res 21:1–16

Nikolova V, Petrova S, Petkova V, Pavlova A, Georgieva T (2007) Antioxidant effects of rooibos tea on workers occupationally exposed to lead. Toxicol Lett 172(S):120–121

Ou B, Hampsch-Woodill M, Prior RL (2001) Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. J Agric Food Chem 49:4619–4626

Pantsi WG, Marnewick JL, Esterhuyse AJ, Rautenbach F, Van Rooyen J (2011) Rooibos (*Aspalathus linearis*) offers cardiac protection against ischaemia/reperfusion in the isolated rat heart. Phytomedicine 18:1220–1228

Persson IA-L, Persson K, Hägg S, Anderson RGG (2010) Effects of green tea, black tea and rooibos tea on angiotensin-converting enzyme and nitric oxide in healthy volunteers. Publ Health Nutr 13(5):730–737

Persson IA-L (2011) The pharmacological mechanism of angiotensin-converting enzyme inhibition by green tea, rooibos and enalaprilat—a study on enzyme kinetics. Phytother Res 26(4):517–521. doi:10.1002/ptr.3588

Petrova A, Davids LM, Rautenbach F, Marnewick JL (2011) Photoprotection by honeybush extracts, hesperidin and mangiferin against UVB-induced skin damage in SKH-1 mice. J Photochem Photobiol 103:126–139

Pietta P-G (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042

Prior RL, Gu L, Wu X, Jacob RA, Sotoudeh G, Kader AA, Cook RA (2007) Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter in vivo antioxidant status. J Am Coll Nutr 26:170–181

Rabe C, Steenkamp JA, Joubert E, Burger JFW, Ferreira D (1994) Phenolic metabolites from rooibos tea (*Aspalathus linearis*). Phytochem 35:1559–1565

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26:1231–1237

Reynecke J, Coetzee WHK, Bester JJA (1949) Rooibos tea. A preliminary report on the composition. Farming South Africa 24:409–412

Sauter W (2004) Vergleich antioxidativer eigenschaften von extrakten aus *Camellia sinensis*, *Rosmarinus officinalis*, *Cyclopia genistoides*, *Cyclopia sessiliflora* and *Aspalathus linearis*. Ph. D. dissertation. Technischen Universitat Munchen, Weihenstephan

Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros- Zevallos L, Jacobo-Velázquez DA (2013) The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal. Methods 5:5990–5999

Schulz H, Joubert E, Schütze W (2003) Quantification of quality parameters for reliable evaluation of green rooibos (*Aspalathus linearis*). Eur Food Res Technol 216:539–543

Skindo Y, Kato K (1991) Effect of rooibos tea on some dermatological diseases. In: Proceedings of the International Symposium on Tea Science, The Organizing Committee of ISTS, Shizuoka, pp. 385–389

Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

Sinisalo M, Enkovaara A-L, Kivistö KT (2010) Possible hepatotoxic effect of rooibos: a case report. Eur J Clin Pharmacol 66:427–428

Sissing L, Marnewick J, De Kock M, Swanevelder S, Joubert E, Gelderblom W (2011) Modulating effects of rooibos and honeybush herbal teas on the developing of esophageal papillomas in rats. Nutr Cancer 63(4):600–610

Snijman P, Joubert E, Ferreira D, Li X-C, Ding Y, Green IR, Gelderblom WCA (2009) Antioxidant activity of the dihydrochalcones aspalathin and nothofagin and their corresponding flavones in relation to other rooibos (*Aspalathus linearis*) flavonoids, epigallocatechin gallate and trolox. J Agric Food Chem 57:6678–6684

Stalmach A, Mullen W, Pecorari M, Serafini M, Crozier A (2009) Bioavailability of C-linked dihydrochalcone and flavanone glucosides in humans following ingestion of unfermented and fermented rooibos teas. J Agic Food Chem 57:7104–7111

Standley L, Winterton P, Marnewick JL, Gelderblom WCA, Joubert E, Britz TJ (2001) Influence of processing stages on antimutagenic and antioxidant potentials of rooibos tea. J Agric Food Chem 49:114–117

Steenkamp A, Fernandes AC, Van Rensburg CEJ (2004) Antioxidant scavenging potential of South African export herbal teas. S Afr J Bot 70:660–663

Ueda S, Nakamura H, Masutani H, Sasada T, Takabayashi A, Yamaoka Y, Yodoi J (2002) Baicalin induces apoptosis via mitochondrial pathway as prooxidant. Mol Immunol 38:781–791

Uličná O, Greksák M, Vančová O, Zlatos L, Galbavy S, Božek P, Nakano M (2003) Hepatoprotective effect of rooibos tea (*Aspalathus linearis*) on CCl_4-induced liver damage in rats. Physiol Res 52:461–466

Uličná O, Vančová O, Božek P, Carsky J, Sebekova K, Boor P, Nakano M, Greksák M (2006) Rooibos tea (*Aspalathus linearis*) partially prevents oxidative stress in streptozotocin-induced diabetic rats. Physiol Res 55:157–164

Uličná O, Vančová O, Waczulikova I, Božek P, Janega P, Babál P, Líšková S, Greksák M (2008) Does rooibos tea (*Aspalathus linearis*) support regeneration of rat liver after intoxication by carbon tetrachloride? Gen Physiol Biophys 27:179–186

Utter AC, Quindry JC, Emerenziani GP, Valiente JS (2010) Effects of rooibos tea, bottled water and a carbohydrate beverage on blood and urinary measures of hydration after acute dehydration. Res Sport Med 18:85–96

Van der Merwe JD, Joubert E, Manley M, De Beer D, Malherbe CJ, Gelderblom WCA (2010) In vitro hepatic biotransformation of aspalathin and nothofagin, dihydrochalcones of rooibos (*Aspalathus linearis*) and assessment of metabolite antioxidant activity. J Agric Food Chem 58:2214–2220

Van Heerden FR, Van Wyk BE, Viljoen AM, Steenkamp PA (2003) Phenolic variation in wild populations of *Aspalathus linearis* (rooibos tea). Biochem Syst Ecol 31:885–895

Villaño D, Pecorari M, Testa MF, Raguzzini A, Stalmach A, Crozier A (2010) Unfermented and fermented rooibos teas (*Aspalathus linearis*) increase plasma total antioxidant capacity in healthy humans. Food Chem 123:679–683

Von Gadow A, Joubert E, Hansmann CF (1997a) Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. Food Chem 60:73–77

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

Von Gadow A, Joubert E, Hansmann CF (1997c) Effect of extraction time and additional heating on the antioxidant activity of rooibos tea (*Aspalathus linearis*) extracts. J Agric Food Chem 45:1370–1374

Wanjiku SM (2009) Antioxidant status of South African beverages and its role on the chemical parameters in human blood. M.Tech. (Biomedical Technology) Thesis, Cape Peninsula University of Technology, Bellville

Yoo KM, Lee CH, Lee H, Moon B-K, Lee CY (2008) Relative antioxidant and cytoprotective activities of common herbs. Food Chem 106:929–936

Yoshikawa T, Naito Y, Oyamada H, Ueda S, Tanigawa T, Takemura T, Sugino S, Kondo M (1990) Scavenging effects of *Aspalathus linearis* (Rooibos tea) on active oxygen species. In: Emerit I, Packer L, Auclair C (eds) Antioxidants in therapy and preventive medicine. Plenum Press, New York