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Ameliorative effect of aspalathin from rooibos (*Aspalathus linearis*) on acute oxidative stress in *Caenorhabditis elegans*

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ABSTRACT

Rooibos leaves and fine stems (*Aspalathus linearis*; Fabaceae) are increasingly enjoyed as herbal tea, largely in fermented (oxidised) red-brown form, but also in unfermented (unoxidised) green form. Rooibos is rich in antioxidant polyphenols, with the dihydrochalcone, aspalathin, as a major active ingredient. We used *Caenorhabditis elegans* as model organism to investigate the effect of rooibos extracts against oxidative stress *in vivo*. In a high glucose environment, *C. elegans* treated with rooibos extract exhibited an extended lifespan. Furthermore, green rooibos was a more potent antioxidant than red rooibos, probably due to its substantially higher aspalathin content. In addition, rooibos decreased acute oxidative damage caused by the superoxide anion radical generator, juglone, with aspalathin playing a major role in improving the survival rate of *C. elegans*. Quantitative real-time PCR results demonstrated that aspalathin targets stress and ageing related genes, reducing the endogenous intracellular level of ROS. These findings suggest that rooibos increases stress resistance and promotes longevity under stress, probably mediated *via* a regulation of the DAF-16/FOXO insulin-like signalling pathway, supporting some of the health claims put forward for rooibos tea.

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Introduction

Reactive oxygen species (ROS) are generated not only by endogenous metabolic functions but also by environmental stimuli such as ultraviolet light or other kinds of radiation. Excess free radicals disturb the dynamic balance between oxidants and antioxidative defence in organisms, leading to oxidative stress which can induce damage to proteins, biomembranes and DNA (Finkel and Holbrook, 2000). Oxidative stress is assumed to be involved in several health disorders, including diabetes, cardiovascular problems and neurodegenerative diseases such as Alzheimer's disease. Therefore, drugs with antioxidant properties are employed in medicine to reduce cellular stress and, in consequence, combat several severe diseases. Medicinal plants, rich in antioxidant polyphenols and terpenoids, could play an important role in this context (Van Wyk and Wink, 2004).

Rooibos (*Aspalathus linearis*), a traditional herbal tea from South Africa, is becoming increasingly popular as a health beverage. Since 2003, exports exceeded 5000 metric tonnes per annum and the product is currently sold in more than 37 countries (Joubert and De Beer, 2011). The cut leaves and young stems are subjected to "fermentation", an oxidative process resulting in a colour change from green to red-brown, hence the final product is also referred to as fermented rooibos or red rooibos. To date studies on the phenolic oxidative changes have focussed on the conversion of aspalathin, a C—C linked dehydrochalcone glucoside (Fig. 1) unique to rooibos, to the flavones, orientin and isoorientin *via* unstable flavanones, and the formation of dimers (reviewed by Joubert and De Beer, 2011) and coloured dibenzofurans (Heinrich et al., 2012). This susceptibility of aspalathin to oxidation results in a substantial reduction in its content in fermented rooibos (Joubert, 1996). Unfermented rooibos, on the other hand, is processed in such a manner as to minimise oxidation of its polyphenols and to retain its green colour; it is accordingly termed green rooibos (Schulz et al., 2003; Joubert and De Beer, 2011). Oxidation of the polyphenols in the course of fermentation and other processing methods leads to weaker antioxidative capabilities in red rooibos (Joubert et al., 2008). Aspalathin is not only the most abundant flavonoid in green rooibos, but it is one of its most potent radical scavengers (Von Gadow et al., 1997; Joubert et al., 2004; Krafczyk et al., 2009; Snijman et al., 2009). Japanese researchers were the first to report an anti-ageing effect on human skin (Joubert and De Beer, 2011).

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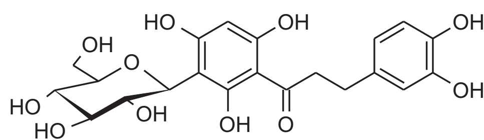


Fig. 1. Structure of aspalathin (2′,3,4,4′,6′-pentahydroxy-3-C-β-D-glucopyranosyldihydrochalcone).

Juráni et al. (2008), using Japanese quails as ageing model, showed that rooibos, when fed to hens, hens contributed to the slowing of age-related decrease in egg production. Furthermore, other beneficial properties such as antimutagenic (Snijman et al., 2007), anti-inflammatory (Baba et al., 2009) and antidiabetic (Kawano et al., 2009) effects have been attributed to aspalathin and rooibos. Detailed summaries of *in vitro* and *in vivo* studies on the bioactivity of rooibos infusions and extracts, as well as its major flavonoids, were presented by Joubert et al. (2008) and Joubert and De Beer (2011).

Caenorhabditis elegans is a tiny free-living nematode, which is widely used as a model organism in different research fields. Due to its small size and short life span it has become an important model for ageing studies, which are facilitated by a large number of transgenic mutants expressing disease-related phenotypes (Hekimi and Guarente, 2003). For instance, *daf-2* encoding an insulin receptor negatively regulates the fork head (FOXO) transcription factor DAF-16 in the insulin/IGF signalling pathway that is involved in metabolic diseases in humans (Kimura et al., 1997).

In this study, we investigated whether rooibos aqueous extract can protect *C. elegans* against oxidative stress caused by the pro-oxidant juglone or a high glucose environment. Our results suggest that aspalathin, as the main compound from green rooibos, can increase the life span of *C. elegans* under stress conditions through the insulin/IGF-1 signalling pathway and enhance oxidative stress resistance by up-regulating the expression of stress-response related genes.

Materials and methods

Preparation of rooibos tea extracts

Extracts were prepared from commercially used plant material (leaves and fine stems) by soaking 150 g of ground plant material in 1.5 l of boiling water, and leaving it to stir overnight (unheated), followed by filtration through cotton wool the next day. The filtrates were then freeze-dried.

High performance liquid chromatography of rooibos tea extracts

HPLC-DAD analysis was carried out as described by Beelders et al. (2012), using an Agilent 1200 system (Agilent, Santa Clara, CA, USA). Gradient separation was performed at 37 °C on a 100 × 4.6 mm 1.8 μm Agilent Zorbax SB-C18 column protected with an Acquity UPLC in-line filter (Waters, Milford, MA, USA) and a 5.0 μm SB-C18 guard column (Agilent) at a flow rate of 1.0 ml/min. Gradient solvents were 2% (m/v) acetic acid in water and acetonitrile. Stock solutions of the phenolic standards were prepared in dimethylsulphoxide (DMSO) and working mixtures of the standards were diluted with water containing ascorbic acid (final concentration *ca.* 10 mg/ml) as antioxidant to stabilize the compounds during analysis. Aspalathin, nothofagin and PPAG (enolic phenylpyruvic acid-2-O-glucoside) were quantified at 288 nm, while orientin, isoorientin, vitexin, isovitexin, hyperoside, isoquercitrin, rutin, quercetin-3-*O*-robinobioside (as rutin equivalents), luteolin-7-*O*-glucoside and ferulic acid were quantified at 350 nm using 5-point calibration mixtures. Extracts were dissolved in water (water-based extracts) at *ca.* 2 mg/ml and diluted with water containing ascorbic acid. Standard mixtures and samples were filtered using 0.22 μm pore-size Millex-GV syringe filtre devices (Millipore) prior to HPLC analysis. Peaks were identified by comparing retention times and UV–vis spectra with those of authentic standards and by comparison to relative retention times reported by Beelders et al. (2012).

Aspalathin and phenolic phenylpyruvic acid-2-*O*-glucoside (PPAG) were obtained from the compound library of ARC Infruitec-Nietvoorbij, Stellenbosch (>95% purity; HPLC-DAD; LC–MS). Rutin and ferulic acid were supplied by Sigma–Aldrich (St. Louis, MO, USA). Other authentic phenolic reference standards (>95% purity) were supplied by Extrasynthese (Genay, France).

Caenorhabditis elegans strains and maintenance

Wild type N2, TK22 (*mev-1(kn1)*) and TJ375 (*hsp-16.2::GFP(gpIs1)*) strains were used in the experiments. All strains were maintained at 20 °C on standard nematode growth medium (NGM) with living *Escherichia coli* OP50 as food source. Age-synchronized worms were obtained by the sodium hypochlorite method (Stiernagle, 2006) cultured within 35 mm × 10 mm Petri dishes (CELLSTAR®) at 20 °C.

Intracellular ROS levels in C. elegans

The fluorogenic probe, 2,7-dichlorodihydrofluorescein diacetate (H_2DCF-DA) (Lebel et al., 1992), was used as indicator to detect and quantify the intracellular ROS level in *C. elegans*. Wild type and *mev-1(kn1)* strains were employed in this assay. Synchronized worms were treated with rooibos tea extracts (100 μg/ml) or aspalathin (0, 10, 20, 50 μM) for 72 h from day 1 after hatching. 50 worms per group were picked up into 100 μl PBS with 1% Tween-20, sonicated and transferred into 96-well plates containing 50 μM H_2DCF-DA. The fluorescence was recorded every 10 min for 2.5 h at 37 °C in a Tecan Safire2™ Microplate Reader (Männedorf, Switzerland) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm (Gutierrez-Zepeda et al., 2005; Abbas and Wink, 2009).

Life span assay

Synchronized worms (3 days old) were transferred to NGM plates or liquid S-Basal medium Petri dishes containing green rooibos extract (100 μg/ml), red rooibos extract (100 μg/ml), or different concentrations of aspalathin (0, 10, 20 and 50 μM) to establish the whole life span of the nematodes, with living *E. coli* as food source. 50 mM glucose (high glucose) was added to NGM plates to attain concentrations similar to those found in poorly controlled diabetes patients (Schlotterer et al., 2009). Adult nematodes were transferred to new Petri dishes (plus ingredients) every day to eliminate the influence of their progeny till the end of the reproductive period, whereafter they were transferred every second day. The number of living worms was counted every day and death was recorded if a worm failed to respond to a gentle physical stimulus. Statistical comparison using a log-rank test was performed with StatView 5.0 software (SAS Institute, Cary, NC, USA).

Survival assay under juglone-induced oxidative stress

High concentrations of juglone, which is a generator of ROS, have a damaging effect on cells and organisms. Age-synchronized wild type N2 worms were treated with green rooibos extract (100 μg/ml), red rooibos extract (100 μg/ml) or aspalathin (0, 10,

20 and 50 μM) for 72 h from the first day after hatching in S-Basal medium with living *E. coli* OP50. Approximately 50 worms per group were transferred into new wells containing 400 μM of juglone. After 6 h exposure to acute oxidative damage, the survivors were examined and scored.

Fluorescence microscopy

The transgenic strain *hsp-16.2::GFP(gpIs1)* was employed in this assay. Age-synchronized L1 larvae were incubated in S-Basal medium containing green rooibos extract (100 μg/ml), red rooibos extract (100 μg/ml) or aspalathin (20 μM) for 48 h, followed by exposure to 20 μM juglone for 24 h. Green fluorescent protein (GFP) is expressed in the pharynx of the nematodes during periods of stress conditions (Rea et al., 2005). At least 20 worms were placed on microscopy glass slides in a drop of PBS containing a paralyzing agent, 10 mM sodium azide. The degree of *hsp-16.2::GFP* fluorescence was measured by fluorescence microscopy at constant exposure times of 400 ms (Nikon-eclipse 90i(2), Nikon Imaging Center, Heidelberg University).

Quantitative real-time PCR

Wild type N2 L1 larvae were treated with up to 20 μM aspalathin for 72 h. Then, the total RNA was isolated with TRIzol reagent (Invitrogen), and was reverse transcribed to cDNA with an ImProm-II™ Reverse Transcription system kit (Promega) following user instructions. Quantitative real-time PCR was executed in a LightCycler (Roche) using SYBR Green Capillary Kit (Abgene).

The primers for quantitative real-time PCR were as follows:
Actin (as housekeeping gene)
(F) 5′-GTG TGA CGA CGA GGT TGC CGC TCT TGT TGT AGA C-3′
(R) 5′-GGT AAG GAT CTT CAT GAG GTA ATC AGT AAG ATC AC-3′

sod-3
(F) 5′-AGC ATC ATG CCA CCT ACG TGA-3′
(R) 5′-CAC CAC CAT TGA ATT TCA GCG-3′

daf-16
(F) 5′-TTT CCG TCC CCG AAC TCA A-3′
(R) 5′-ATT CGC CAA CCC ATG ATG G-3′

Statistical analyses

All data come from three independent sets of experiments, unless mentioned otherwise. A two-tailed unpaired Student's *t*-test was performed to compare two groups, while comparisons between multiple groups were executed by one-way analysis of variance (ANOVA) followed by *post hoc* analysis with Tukey's test (Prism, GraphPad Software, San Diego, CA).

Results

Phenolic content of rooibos extracts

HPLC-DAD chromatograms of the green and red rooibos extracts at 288 and 350 nm are depicted in Fig. 2. Quantitative data are summarised in Table 1. As expected, aspalathin was the main compound in green rooibos extract (34.66 mg/g). The other compounds were present at much lower concentrations. Of these isoorientin, orientin and PPAG were present at ≤4.8 mg/g extract, and nothofagin, quercetin-3-*O*-robinobioside and rutin were present at ≤2.2 mg/g extract. The remaining compounds each comprised less than 1 mg/g extract, while no ferulic acid was detected. The red rooibos extract contained higher levels of all the compounds, except aspalathin, nothofagin, rutin and luteolin-7-*O*-glucoside. Ferulic acid was present in the red rooibos extract.

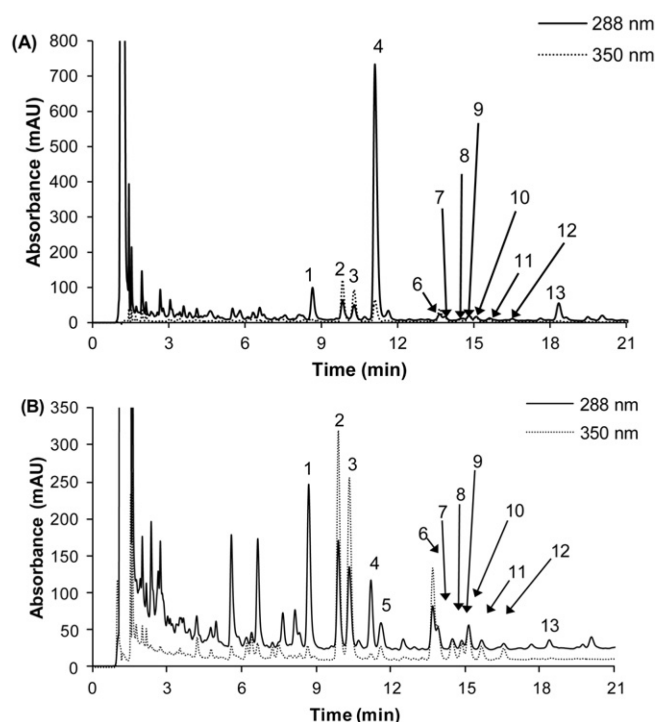


Fig. 2. HPLC-DAD chromatograms of green rooibos (A) and red rooibos (B) extract at 288 and 350 nm. Compounds are PPAG (1), isoorientin (2), orientin (3), aspalathin (4), ferulic acid (5), quercetin-3-*O*-robinobioside (6), vitexin (7), hyperoside (8), rutin (9), isovitexin (10), isoquercitrin (11), luteolin-7-*O*-glucoside (12) and nothofagin (13). PPAG is phenylpyruvic acid-2-*O*-glucoside.

Rooibos attenuates the intracellular ROS level in C. elegans

Using H_2DCF-DA to measure the endogenous intracellular ROS level, ROS scavenging ability was indicated for rooibos in *C. elegans*. Treatment of wild type *C. elegans* with rooibos resulted in a decreased ROS level, diminished by 30.6% ($p < 0.01$) for green rooibos, and by 19.8% ($p < 0.05$) for red rooibos, compared to the control (Fig. 3 A). In comparison to red rooibos, the lower level of ROS in *C. elegans* pretreated with green rooibos can probably be attributed to its higher content of aspalathin. We therefore measured intracellular ROS levels in *C. elegans* pretreated with aspalathin. The result showed that 20 and 50 μM aspalathin attenuated the level of ROS by up to 34.8% ($p < 0.01$) and 47.4% ($p < 0.001$), respectively, in wild type *C. elegans* (Fig. 3B), and by 43.1% and 42.3% ($p < 0.01$),

Table 1
Individual polyphenol content of rooibos extracts.

Class	Flavonoid	Green rooibos (mg/g)	Red rooibos (mg/g)
Dihydrochalcone	Aspalathin	34.66	2.01
	Nothofagin	2.18	0.27
Flavone	Isoorientin	4.80	6.01
	Orientin	4.03	5.25
	Isovitexin	0.47	0.73
	Vitexin	0.61	1.02
	Luteolin-7-*O*-glucoside	0.49	0.37
Flavonol	Quercetin-3-*O*-robinobioside	1.92	3.05
	Hyperoside	0.53	0.86
	Rutin	2.09	0.79
	Isoquercitrin	0.64	0.67
Other	PPAG	3.22	3.67
	Ferulic acid	nd	0.59

PPAG (phenyl pyruvic acid-2-*O*-glucoside); nd – not detected.

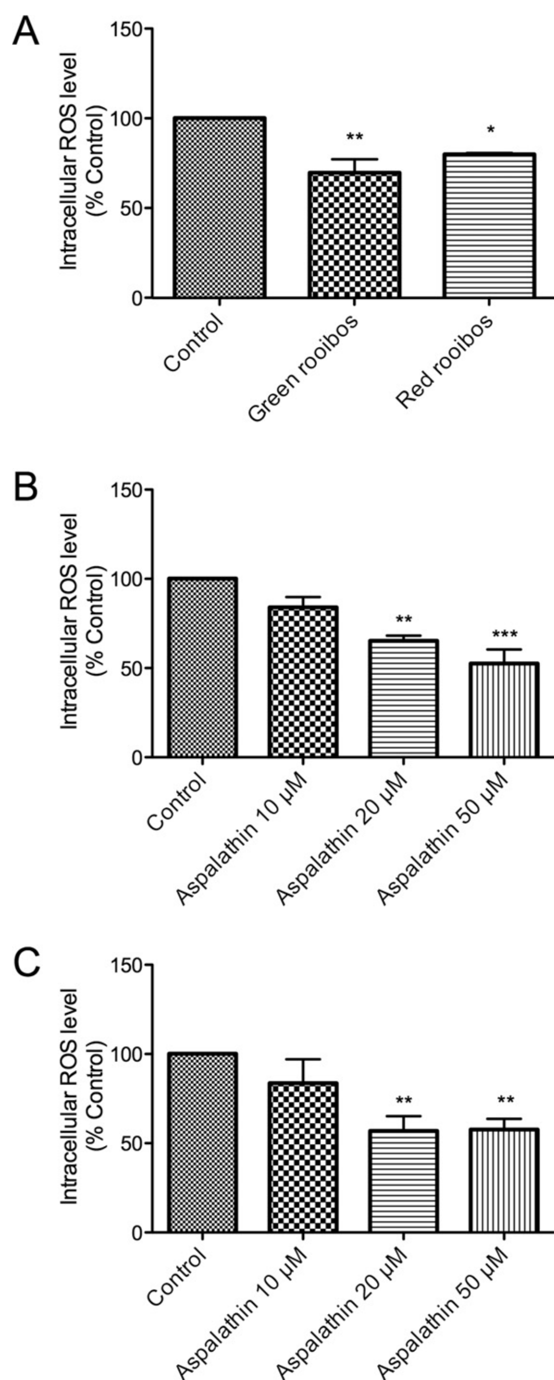


Fig. 3. Dose dependence of rooibos extracts (A) and aspalathin on ROS levels in wild type N2 (B) and *mev-1* mutant strain (C). After 72 h of treatment, 50 worms for each group were collected and analysed in the H_2DCF-DA assay. Values are means ± SE from three independent experiments: $^{**}p < 0.01$, $^{***}p < 0.001$.

respectively, in *mev-1* mutant strain worms (Fig. 3C), indicating that aspalathin from green rooibos plays a key role in attenuating intracellular ROS levels *in vivo*.

Rooibos extends the life span of C. elegans under glucose-induced oxidative stress

Age-synchronized worms were fed with green or red rooibos extract for their entire lifespan under high glucose conditions. The results show that green rooibos exhibits a substantial protective effect against oxidative stress under high glucose concentration, increasing the life span of *C. elegans* by 22.5% (Fig. 4A), with a corresponding value of 14.0% for the red rooibos group. The question was whether aspalathin by itself can also alleviate the damages of glucose-induced oxidative stress. We found that aspalathin extended the life span of *C. elegans* in a dose-depended manner under high glucose conditions. The mean life span was significantly extended by 24.4% and 20.5% in groups treated with 20 μM and 50 μM aspalathin, respectively (Fig. 4B). On the other hand, we did not observe any life span extension under rooibos and aspalathin treatment and normal culture conditions (Fig. 4C and D).

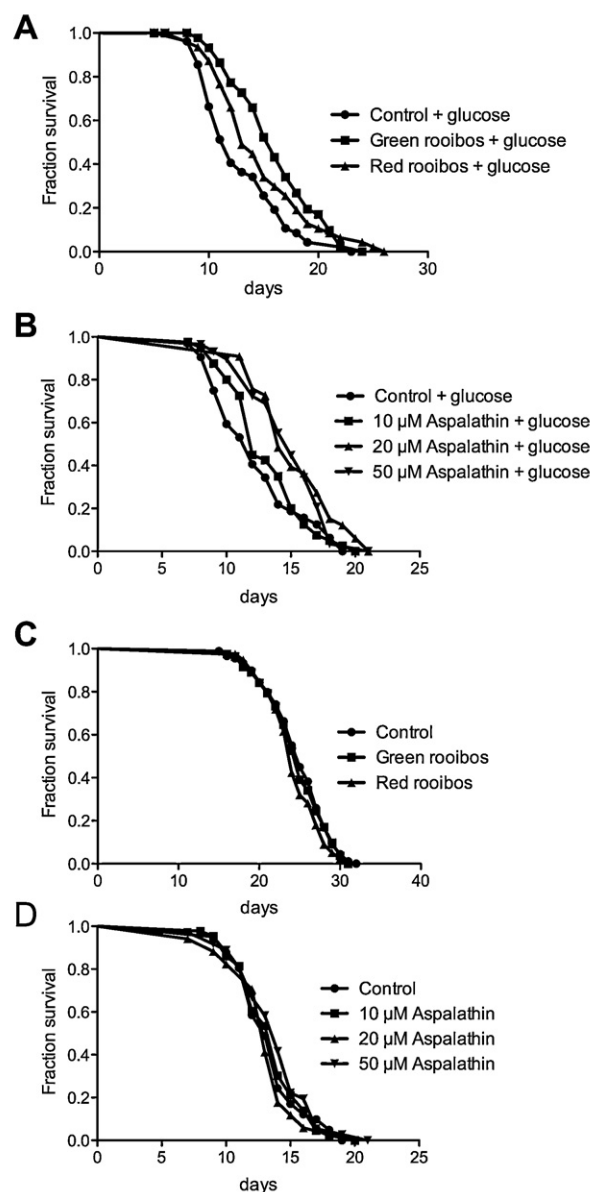


Fig. 4. Rooibos (A) and aspalathin (B) extend the life span of wild type *C. elegans* under high glucose conditions, whereas no extension was detectable under normal culture conditions (C and D).

Rooibos increases the stress resistance in C. elegans

Age-synchronized L1 larvae were treated with rooibos extracts or aspalathin for 72 h to estimate survival rate, scored as viability after exposure to a lethal dose of the prooxidant juglone for 6 h. Green rooibos extract was more potent against oxidative stress than red rooibos extract (Fig. 5A). The survival rate was increased by 106% in the green rooibos group, and only by 45.9% in the red

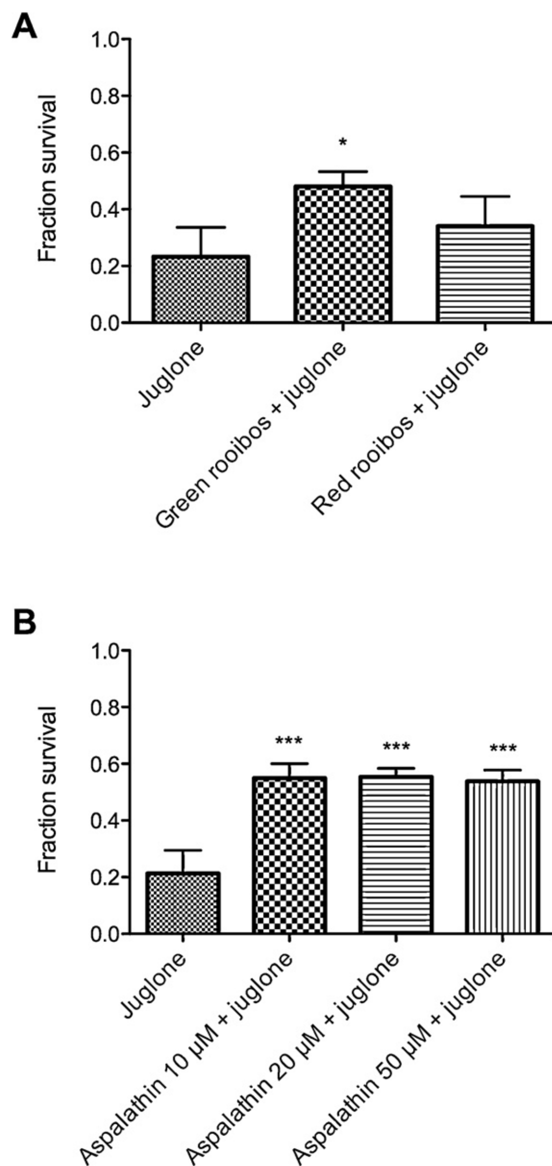
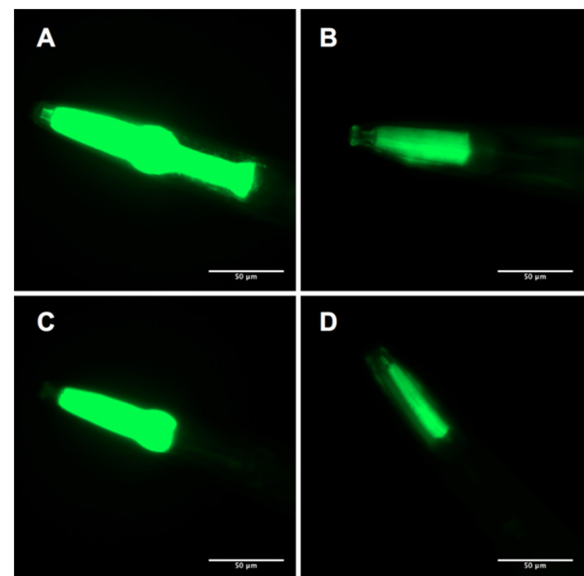


Fig. 5. Rooibos extracts (A) and aspalathin (B) protect wild type *C. elegans* against acute oxidative stress induced by the pro-oxidant juglone. Age-synchronized worms were treated with green rooibos ($n = 150$), red rooibos ($n = 147$) extract or different concentrations of aspalathin (10 μM, $n = 216$; 20 μM, $n = 219$; 50 μM, $n = 187$), control ($n = 360$) for 72 h from the first day after hatching. Subsequently, they were exposed to 400 μM juglone for 6 h. Number of living nematodes were assessed. Data are means ± SE. Statistical significance, $^{*}p < 0.05$; $^{***}p < 0.001$.



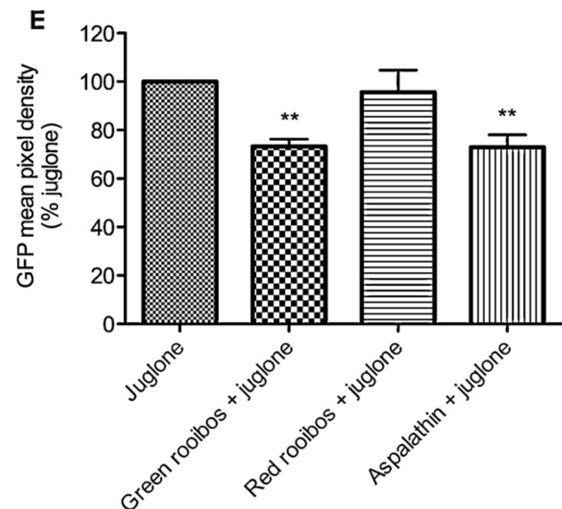


Fig. 6. Rooibos extracts and aspalathin suppress *hsp 16.2* gene expression induced by juglone in *hsp-16.2::GFP(gpIs1)* strain. Under normal conditions, GFP expression is not detectable. After induction by juglone, GFP was expressed in the pharynx (A); pre-treatment with green rooibos (B), red rooibos (C) or aspalathin (D) inhibited GFP expression; (E) quantification of GFP expression. Data were from three independent experiments with at least 25 worms in each group. Data are presented as means ± SE. Statistical significance, $^{**}p < 0.01$.

rooibos group (not significant), when compared to the untreated control group. The corresponding effect of purified aspalathin was recorded as well; survival was significantly ($p < 0.001$) enhanced by 158.2% at 10 μM, 160.1% at 20 μM and 152.6% at 50 μM aspalathin, respectively (Fig. 5B).

To further evaluate the protective effect of rooibos against oxidative stress, the transgenic strain *hsp-16.2::GFP(gpIs1)* was employed in the experiments. No fluorescence can be detected in nematodes under normal culture conditions, while a strong GFP expression in the pharynx is attributed to juglone-induced oxidative stress (Fig. 6A–D). The fluorescence density in the pharynx of *C. elegans* was reduced by 26.7% ($p < 0.01$) in the group pretreated with 100 μg/ml green rooibos, but only by 4.3% for the group pretreated with 100 μg/ml red rooibos (Fig. 6E), both compared to the untreated group. In addition, aspalathin also significantly decreased the *hsp-16.2::GFP* expression by 27.0% ($p < 0.01$) (Fig. 6E). This demonstrates again the effect of aspalathin on stress resistance in *C. elegans*.

Aspalathin increases expression of stress response related genes in C. elegans

To investigate mechanism of action of aspalathin, the expression of the stress response related genes *daf-16* and *sod-3* was examined by quantitative real-time PCR. Treatment with aspalathin significantly increased the expression of *daf-16* and *sod-3* 1.2 fold ($p < 0.05$) and 4.2 fold ($p < 0.001$), respectively (Fig. 7).

Discussion

Free radicals create an imbalance between oxidants and the endogenous antioxidant defence system thereby contributing to oxidative stress which is a major factor in many human diseases. Antioxidant compounds are renowned for their free radical

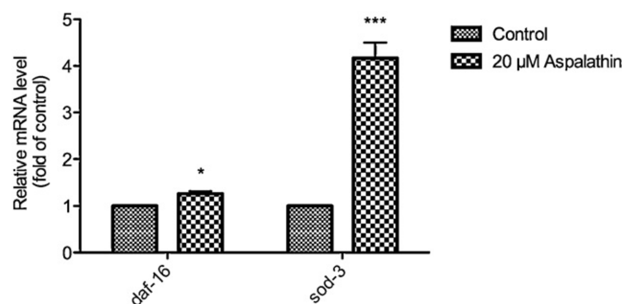


Fig. 7. Quantification of relative expression of *daf-16* and *sod-3* in aspalathin-treated worms. The expression of *actin* gene was used as an internal reference. Data are means ± SE. $^{*}p < 0.05$, $^{***}p < 0.001$.

scavenging activity, which helps to attenuate oxidative stress not only *in vitro* but also *in vivo*. More and more natural products are found to possess these antioxidative properties. Rooibos, a traditional medicinal plant from South Africa, has been reported to have cardioprotective effects and mitigate metabolic disorders, probably due to its high content of polyphenols (Beltran-Debon et al., 2011; Pantsi et al., 2011). In our work, we investigated the protective properties of both green and red rooibos aqueous extract in *C. elegans*, a model invertebrate organism used to study ageing and longevity. The major difference between green and red rooibos extracts is the high level of aspalathin in green rooibos. Although the same plant material was not used for preparation of green and red rooibos and large natural variation exists between different batches of plant material due to use of seedlings, certain trends regarding their composition were similar to other studies (Bramati et al., 2003; Joubert et al., 2005; Beelders et al., 2012). Importantly, the flavone analogues of aspalathin, isoorientin and orientin, were higher in the red rooibos extract than the green rooibos extract, partly attributed to the role of fermentation in their formation and the plant material used. Our results clearly show that rooibos and aspalathin attenuate the intracellular level of hydrogen peroxide anions and other ROS, indicating that the rooibos polyphenols or their metabolites are being absorbed by worms. In addition, it has been reported that aspalathin is absorbed in mammals (Kreuz et al., 2008). Its presence has been reported in human plasma and urine after consumption of rooibos beverage (Stalmach et al., 2009; Breiter et al., 2011). Other antioxidant substances, such as *Ginkgo biloba* extract EGb761 (Strayer et al., 2003), soy isoflavone (Gutierrez-Zepeda et al., 2005) and EGCG from green tea (Abbas and Wink, 2009) have similar properties and also reduce ROS levels in *C. elegans*. Furthermore, *mev-1(kn1)* mutants overproduce free radicals (Ishii, 2000), whose level can be significantly lowered through a pretreatment with aspalathin (Fig. 3C), indicating again the compound's effect *in vivo*.

Glucose decreases the life span of *C. elegans* by inhibiting the FOXO transcription factor DAF-16 (Lee et al., 2009). In our experiment, the expression of *daf-16* genes was upregulated after aspalathin pretreatment (Fig. 7). It is therefore likely that aspalathin exerts its effect through an insulin/insulin-like signalling pathway to extend the lifespan of *C. elegans* under high glucose conditions (Fig. 4B). It has been suggested that *C. elegans* is suitable for diabetic research if the hyperglycemic conditions in diabetes patients are mimicked by creating a high glucose environment for the worms (Schlotterer et al., 2009). Aspalathin was reported to increase glucose uptake and insulin secretion in previous research (Kawano et al., 2009). Our data also showed aspalathin to extend the life span of *C. elegans* under high glucose-induced oxidative stress, demonstrating that aspalathin, albeit a natural compound, has potent antidiabetic abilities.

Rooibos is apt at preventing oxidative damage *in vivo*. Juglone increases the production of intracellular $O_2^{\bullet -}$ and H_2O_2 by affecting the function of the electron transport chain and DNA (Hassan and Fridovich, 1979; Strayer et al., 2003). When worms are exposed to a high dose of juglone, the resulting intracellular superoxide anion radicals function as toxins, as shown before by other authors (De Castro et al., 2004; Burmeister et al., 2008). Green rooibos and aspalathin can prevent juglone toxicity, as evidenced by increased survival rate. Scavenging of superoxide anion radicals has been demonstrated for aspalathin and other rooibos flavonoids. It was also found to be more effective than isoorientin and orientin (Joubert et al., 2004). Red rooibos has little protective effect against acute stress, probably due to its lower amount of polyphenols, especially aspalathin. During the fermentation process nothofagin, another potent antioxidant dihydrochalcone (Snijman et al., 2009) and a major constituent of green rooibos, is oxidised (Joubert, 1996). Fermentation results in a loss in antioxidant activity of rooibos (Joubert et al., 2008). The modulated GFP expression in *hsp-16.2::GFP(gpIs1)* mutants (Fig. 6E) also suggests that rooibos protects against acute oxidative damage. Small heat shock proteins (sHSPs) influence stress resistance through the insulin/IGF-1 signalling pathway (Hsu et al., 2003). Our results also demonstrate that aspalathin increased stress resistance *in vivo*, leading to an improved performance of *C. elegans* under acute oxidative stress, probably working similarly through this insulin/IGF-1 signalling pathway.

On the other hand, neither rooibos nor aspalathin promoted life span expansion of *C. elegans* under normal culture conditions (Fig. 4C and D). Quantitative real-time PCR results indicated that aspalathin up-regulates the expression of *sod-3*, which encodes the enzyme superoxide dismutase (SOD) that eliminates ROS in cells. Some researchers claim that enhanced SOD levels protect against oxidative stress, yet failing to extend the life span of *C. elegans* (Doonan et al., 2008). Our experiments agree with this finding in that aspalathin did not extend the life span of *C. elegans*, while the *sod-3* gene was up-regulated (Fig. 7). The homeostasis of the external or internal production of ROS and the subsequent stress response is related with the ageing process and many age-related diseases (Finkel and Holbrook, 2000). It is suggested that aspalathin might trigger the expression of antioxidative defence genes to protect *C. elegans* against oxidative damage, thus promoting longevity under stress.

In conclusion, aspalathin plays a major role in protecting worms and probably other animals (and humans) against oxidative stress, especially when ROS are enhanced by internal or external stressing agents. Therefore, the consumption of rooibos tea might help prevent health disorders which are related to oxidative stress.

Conflict of interest

The authors declare that no conflict of interest exists.

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