Chapter 16

Rooibos and Honeybush: Recent Advances in Chemistry, Biological Activity and Pharmacognosy

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Rooibos (*Aspalathus linearis* (Brum.f.) Dahlg.) and honeybush *(Cyclopia* Vent. species*)* have a long history of traditional uses in South Africa as indigenous herbal health beverages or tisanes. These herbal teas are rich in several unique polyphenolic compounds that not only differ from that of *Camellia sinensis* teas, but also from one another. The bioactivity of both includes *in vitro* and *in vivo* antimutagenic, antioxidant, cancer modulating, cardiovascular and other activities. The first clinical controlled study which showed that rooibos decreased oxidative lipid damage and increased the redox status in plasma is discussed while human studies on honeybush have not yet been studied to date.

In African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality; Juliani, H. Rodolfo, et al.; ACS Symposium Series; American Chemical Society: Washington, DC, 2010.

Rooibos, *Aspalathus linearis* (Brum.f) Dahlg. (Family Fabaceae; tribe Crotalarieae) indigenous to the Cedarberg and neighboring mountains of the Western Cape Province of South Africa has been used as a herbal beverage by the indigenous Khoi people since the late 1700s (*1*). A Russian immigrant, Benjamin Ginsberg started commercial rooibos trading in 1904. Today rooibos is cultivated on a large scale (more than 12 000 metric tons, http://sarooibos.co.za/content/view) to serve both local and increasing international market demands. Aside from the tea after infusing creating a deep natural red color with a full flavor and body, this herbal tea is caffeine free (*2*), has a low tannin content – estimated at 3% in the leaves (*3,4*), is high in antioxidant activity and bioactive phytochemicals. Scientific studies have shown via *in vitro* and animal laboratory based experiments and newly conducted human studies that this herbal tea will promote good health. Together, all these factors have contributed to the popularity of this herbal tea as a health beverage. Traditionally, rooibos and honeybush are consumed as a hot brew where one cup of freshly boiled water contains one tea bag (~2.4g) with an infusion time of 2-5 min. This brew is then either consumed hot with the addition of milk and/or sugar or cold with the addition of lemon juice and honey.

Unlike rooibos being an established South African crop in the industry, honeybush (*Cyclopia* species) is still in its infancy of developing into a sustainable crop for commercial use, with a current annual production of <300 tons. The species grow within the coastal and mountainous areas of the Western and Eastern Cape Provinces (*5*) being part of the Fynbos biome. More than 20 species have been identified of this woody legume, but the product known as honeybush herbal tea is made from mainly two species, *Cyclopia intermedia* E. May *and C. subternata* Vogel (*6).* Like rooibos, honeybush does not contain caffeine and has a low-tannin content rendering it well suited for a night time beverage and for those experiencing nervousness (*7,8*).

Both rooibos and honeybush species belong to the Fynbos biome, which accounts for more than 80% of the plant species in the Cape Floral Kingdom, the smallest but highest in biodiversity of the six plant kingdoms in the world.

Phytochemistry

During manufacturing, two forms of rooibos are produced, traditional/fermented and "green"/unfermented rooibos. With traditional rooibos the freshly cut leaves and stems are placed purposefully in piles and allowed to ferment as it is during the fermentation process that most of the aromas of the herbal tea as well as the final color develop. The fermented leaves are then dried and it is the art of drying that can significantly impact the quality (time and temperature). In contrast, and similar to the processing of traditional Chinese green tea, in the processing of green rooibos herbal tea, oxidative changes are kept to a minimum with a quick drying of the plant material (*9*). Similar to rooibos, honeybush also needs to be fermented and more recently has also become available in a 'honeybush green tea' form as well. It is during fermentation that the rich red color and unique flavor are developed that is so unique to traditional rooibos, also referred to as "red" tea or "red bush" tea. The

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"green" rooibos does not have this color nor flavor but rather has a green/yellow color with a "grassy" nose.

The phenolic constituents of traditional and green rooibos differ from that of *Camellia sinensis* teas and are unique in that it contains aspalathin, a C-C linked dihydrochalcone glucoside (*10*) and also the recently discovered cyclic dihydrochalcone, aspalalinin (*11*). To date, rooibos is the only source of aspalathin which allows this compound to be used as a chemical marker for quality control in the case of green rooibos and authentication of plant material. Another rare compound, nothofagin, a 3-dehydroxy dihydrochalcone glucoside, previously shown to be found in the heartwood of *Nothofagus fusca* (*12*) is also present in the rooibos teas (*13*). During fermentation (an oxidative environment) the dihydrochalcone content of rooibos decreases substantially with less than 7% of the original aspalathin content remaining in traditional rooibos *(14*) as aspalathin is oxidized to dihydro-iso-orientin (*15*). A recent report showed that during degradation of aspalathin under oxidative conditions, the diastereomeric mixtures of dihydro-iso-orientin and dihydro-oerientin are formed as the major and minor products respectively, with maximum concentrations of iso-orientin and orientin occurring after 6 h (*16*). Several other phenolic compounds are present in rooibos and include the C-C linked β-D-glucopyranosides such as the flavones orientin, iso-orientin, isovitexin and vitexin, and the flavanones dihydro-orientin, dihydro-iso-orientin and hemiphlorin (*16*) which are also degraded during fermentation but to a lesser extent. Other flavonols present in rooibos include hyperoside, quercetin, quercetin-3-roninobioside, rutin, iso-quercitrin and the flavones luteolin, luteolin-7-O-glucoside and chrysoeriol (*13*). The flavanols, (+)-catechin and (-)-epicatechin form the chain extending units of the rooibos procyanidin-type tannin with (+)-catechin as terminal unit (*17*). Variations in the polyphenolic content of rooibos may occur due to genetic variation in plant material when seeds are used for propagation, wild compared to cultivated populations of *A. linearis* and differences in the manufacturing process (*18,19,20*).

The polyphenolic composition of honeybush differs from that of rooibos as well as *Camellia sinensis* teas. The three major constituents in the leaves of the more than 20 *Cyclopia* species investigated by De Nysschen and co-workers (*6*) included the xanthone mangiferin and glycosides of the flavanones hesperetin and isosakuranetin. Combinations of these three compounds in varying quantities are seen as a unique character for *Cyclopia* species, although a recent study using LC-MS to identify polyphenols, could not report on any detectable quantities of isosakuranetin in many of the *Cyclopia* species (*21*). Later studies also identified other phenolic metabolites with potential health benefits such as flavonols, flavonones, isoflavones, coumestans, flavones, xanthones, cinnamic acids and (+)-pinitol, with mangiferin, isomangiferin and hesperedin present in all species analyzed to date (*21,22,23*). The total polyphenol, flavonol/flavone, and flavanol/proanthocyanidin content of a cup (200 mL) of traditional/fermented and green/unfermented rooibos and honeybush herbal teas is presented in Table I (data obtained from Analytical Laboratory, Oxidative Stress Research Centre, CPUT, South Africa). The herbal teas were provided by Rooibos Limited (Mr A Redelinghuys, Clanwilliam, South Africa).

Table I Total Polyphenol, Flavonol and Flavanol Content of a Single Cup[1] of Rooibos and Honeybush Herbal Teas.

Antioxidant content/capacity	*Traditional[2] rooibos*	*Green rooibos*	*Traditional[2] honeybush*	*Green honeybush*
Total polyphenols[b]	73.4 ± 1.8	106.5 ± 2.1	23 ± 0.2	74.0 ± 1.9
Total flavonols/ Flavones[c]	33.4 ± 0.4	26.9 ± 0.3	2.2 ± 0.06	16.4 ± 0.3
Total flavanols/ pro-anthocyanidins[d]	2.6 ± 0.02	6.0 ± 0.1	0.6 ± 0.01	4.3 ± 0.1

NOTE: [1]A single cup = 200 mL, prepared by steeping one tea bag in 200 mL of freshly boiled water for 5 min, [b]the Folin–Ciocalteu method was used to determine the total polyphenol content and is expressed as mg GAE (gallic acid equivalents), [c] mg of quercetin (360 nm method), [d] mg of catechin DMACA method. [2]Traditional = Fermented; Values in columns are means ± SD of 10 samples done in triplicate.

The presence of phenolic acids in rooibos such as caffeic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid and protocatechuic acid have also been reported (*13*). Recently, a study reported on the enhancement of the antioxidant yield and soluble solid matter from fermented rooibos with the addition of a fungal cocktail of hydrolyzing enzymes (*24*). When using green rooibos material, a resultant semi-fermented rooibos is produced with a higher aspalathin content. The influence of the enzyme treatment on the unique aroma of rooibos still needs to be investigated. Previously 99 and 218 volatile components were reported in the vacuum steam distillate and headspace vapor of traditional rooibos (*15*), respectively. Guaiacol (24%), 6-methyl-3,5-heptadien-2 one isomer (5.2%), damascenone (5%), geranylacetone (4.2%), β-phenylethyl alcohol (4.1%) and 6-methyl-5-hepten-2-one (4%) comprises the major volatile components (*25*). A later study reported on a similar characterization of the volatile fraction of traditional/fermented rooibos and concluded that the dichloromethane extraction from a brewed extract consisted of 50 components while the steam distillation and extraction fraction consisted of 123 components, with 42 components were identified as new rooibos volatiles (*26*).

The aroma volatiles of honeybush have also been reported by Wang *et al* (*27*). The aroma components were dominated by monoterpene alcohols, of which α-terpineol (28%) was the major component, with minor amounts of linalool (7%), nerol (2%) and geraniol (8%). These monoterpenes are responsible for the sweet, floral and fruity notes of the tea, while other components such as phenylethyl alcohol (3%) and 5-methylfurfural (2.1%) imparted also sweet and honey notes. Other volatiles such as eugenol (6%), linalool oxides (7%), and methyl-heptenol (3%) were also detected. With both honeybush and rooibos, the exact nature of the aromas and flavor will depend significantly on the species collected, time of collection, drying, fermentation and processing (*27*).

Rooibos and honeybush also contain essential micro- and macro elements, with the traditional/fermented forms containing lower amounts of elements than the "green"/unfermented forms (*28*). The levels of Al and Ni in the rooibos and

honeybush infusions were shown to be significantly lower when compared to that of tea and coffee, while no significant differences in element quantities in rooibos and honeybush infusions could be reported. The elemental content in the raw plant material (1g plant material was decomposed at 500 ^{0}C for 16 h and ash heated at 100 ^{0}C for 5-10 min in 3 mL *Aqua regia* made up to final volume of 20 mL) and in hot water infusions of traditional/fermented and green rooibos and honeybush (50 mL of boiled distilled water was used to extract 1g rooibos material for 15 min) is shown in Table II.

Table II. Mineral Content of Traditional/Fermented and Green Rooibos and Honeybush Plant Material (mg/kg) and Infusions (mg/L).

	Al	*B*	*Ca*	*Cu*	*Fe*	*K*	*Mg*	*Mn*	*Ni*	*P*	*Zn*
[a]Rf-raw	172	20	1926	11	120	3628	1687	51	1.3	325	8
[b]Rg-raw	90	13	4598	6	36	2104	2226	42	0.5	438	5
[c]Hf-raw	80	29	2166	10	62	5272	1146	42	1.3	868	9
[d]Hg-raw	131	38	1206	13	86	2702	495	65	2	1399	20
[a]Rf-inf	0.2	0.4	7	0.2	0.2	58	10	0.2	0.0	2	0.0
[b]Rg-inf	0.2	0.4	19	0.2	0.0[e]	38	20	0.6	0.0	8	0.1
[c]Hf-inf	0.2	0.2	9	0.1	0.1	93	11	0.2	0.0	12	0.1
[d]Hg-inf	0.2	0.3	15	0.1	0.1	105	16	0.4	0.0	12	0.2

NOTE: aRf-raw/inf: fermented rooibos plant material/infusion; bRg-raw/inf: green rooibos plant material/infusion; cHf-raw/inf: fermented honeybush plant material/infusion; dHg-raw/inf: green honeybush plant material/infusion. e 0.0 less than 0.1 All plant materials were bought in a market in Czech Republic.

SOURCE: modified from Malik et al., 2008 (28).

Bio-activity

Traditionally, rooibos has a long history of medicinal use with anecdotal evidence linking the consumption to relief of digestive disorders, skin allergies, insomnia, nervous tension and mild depression (*1,29*). In 1968, a young South African mother, Annetjie Theron, found that rooibos appeared to alleviate her baby's symptoms of colic. She documented all these effects in a book and started to communicate her observations through the press and other public forums (*30*). Since that time, rooibos has been recommended for many other ailments and Annetjie Theron established her own cosmetic and toiletry business in South Africa, distributing worldwide with rooibos being a key ingredient.

Japanese and South African researchers were the first to scientifically investigate the possible health promoting properties of rooibos which led to publications reporting on various biological activities (*31-37*). A few studies regarding the *in vitro, in vivo* and *ex vivo* biological activities of honeybush has been published by mainly South African researchers and are mostly limited to the exported species, *Cyclopia intermedia.* These studies will be discussed.

Honeybush has traditionally been used as an expectorant, a stimulator of milk production in lactating women and to treat various digestive disorders *(29,38,39)* but no clinical trials have been conducted yet.

Antioxidant Properties

Flavonoids have been shown to exhibit powerful antioxidant activities with mechanisms involving free radical scavenging, metal chelation and singlet oxygen quenching with the inhibition of enzyme activity. As previously mentioned, rooibos is rich in flavonoids. Numerous studies have reported on the antioxidant activity of rooibos using various types of extracts of rooibos in a number of different assay systems (*33,34,40-44*). In one of the earliest studies published it was shown that aqueous extracts of traditional rooibos effectively scavenged the superoxide anion radical ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) when using electron spin resonance spectrometry (*33*). Recently a review was published where the antioxidant activities of hot water extracts from traditional/fermented and green/unfermented rooibos were compared using $O_2^{\bullet-}$, $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical), $ABTS^{\bullet+}$ (2,2'-azino-di-3-ethylbenzothiazoline-6-sulfonic) and FRAP (ferric reducing antioxidant potential) assay systems (*20*). These results clearly show that the antioxidant potential decreases as a result of fermentation and could be attributed to a decrease in total polyphenol content. Results from a previously published study confirmed that there is a strong correlation (R^2=0.812) between the aspalathin content in green/unfermented rooibos and the total antioxidant activity as determined by the $ABTS^{\bullet+}$ scavenging method was shown (*45*).

In vitro antioxidant activity for the various *Cyclopia* species can be impacted by processing (*46,47*). The antioxidant activities of a cup of fermented and green rooibos and honeybush (source: Rooibos Ltd., Clanwilliam, South Africa) using the oxygen radical absorbance capacity (ORAC) assay (unpublished data, Dr JL Marnewick and Mr F Rautenbach, Analytical Laboratory, Oxidative Stress Research Centre, CPUT, South Africa) shows that green rooibos and green honeyhush exhibit greater antioxidant activity than the same lot fermented (Table III).

Table III Antioxidant activity of a cup[a] of rooibos and honeybush herbal teas

Antioxidant capacity	*Traditional[c] rooibos*	*Green rooibos*	*Traditional[c] honeybush*	*Green honeybush*
ORAC (μmole TE[b])	1537.6 ± 27	2093.6 ± 50	780.1 ± 20	1620.5 ± 37

NOTE: [a]A cup = 200 mL, prepared by steeping one tea bag in 200 mL of freshly boiled water for 5 min; [b]Antioxidant activity is expressed as μmole trolox equivalents (TE) per cup, using the fluorescein method; [c]Traditional = Fermented; Values in columns mean ± SD of 10 samples done in triplicate.

The use of natural antioxidants in supplements can also exhibit pro-oxidant activity under certain conditions such as the concentration and nature of the polyphenolic compounds causing oxidative damage to important cellular components (*48*). Aqueous extracts and crude polyphenolic fractions of both traditional and green rooibos were evaluated for possible pro-oxidant activity using a Fenton reaction model system containing $FeCl_3$-EDTA and H_2O_2 for the generation of hydroxyl radicals. Pro-oxidant activity was shown for pure aspalathin while the dihydrochalcone and flavonoid contents of the enriched

rooibos extracts correlated (R^2 = 0.977 and 0.971) with the pro-oxidant activity, but the total polyphenol content did not show any correlation (*48*).

All these studies mostly demonstrated *in vitro* antioxidant activities, but recently *ex vivo* and *in vivo* antioxidant capacity of rooibos and honeybush were also shown in various experimental animal studies (*37,49-52*). Being known for its good antioxidant potential *in vitro*, the effect of chronic administration of traditional rooibos on lipid peroxidation in rat brain led to positive results (*37*). After consuming rooibos for 21 months damage to the central nervous system was assessed by measuring the accumulated lipidperoxides and TBARS (thiobarbituric acids reactive substances) in the brains of these female rats. The MRI (magnetic resonance imaging) scans showed a significant lower content of TBARS, comparable to that of young five week old control rats. Of importance is the fact that aging induces deterioration of the CNS (central nervous system) that is partly due to the cytotoxic effect of reactive oxygen species (ROS) generated in the brain, and rooibos suppressed the accumulation of lipidperoxides associated with the aging process (*37*). A recent study used Japanese quails as a model for ageing and reported that the consumption of either a rooibos extract or milled rooibos plant material reduced the decrease in egg production by aged quail hens, thereby prolonging their productive period (*53*), with a possible explanation that rooibos might exhibit estrogenic activity, but this work needs to be reconfirmed or verified by others. These studies do however, suggest and provide a rationale that rooibos herbal tea as viewed by the Japanese are an anti-ageing herbal beverage.

Although, long term consumption of traditional rooibos by Japanese quails did not alter the fragility of erythrocytes to H_2O_2-induced hemolysis, a decreased *ex vivo* peroxide-induced hemolysis of their red blood cells was shown (*49*). When traditional and green rooibos and honeybush were consumed by male Fischer rats for 10 weeks at a concentration customarily used for human consumption (2% w/v), interesting results were obtained in terms of the hepatic oxidative status and drug metabolizing enzymes (*50*). Although none of the rooibos or honeybush herbal teas had any significant effect on the hepatic ORAC (oxygen radical absorbance capacity) values, both rooibos and green honeybush improved the oxidative status in the liver as shown by a significant increase in the ratio of reduced to oxidized glutathione (GSH:GSSG), an indicator used for cellular oxidative stress (Figure 1).

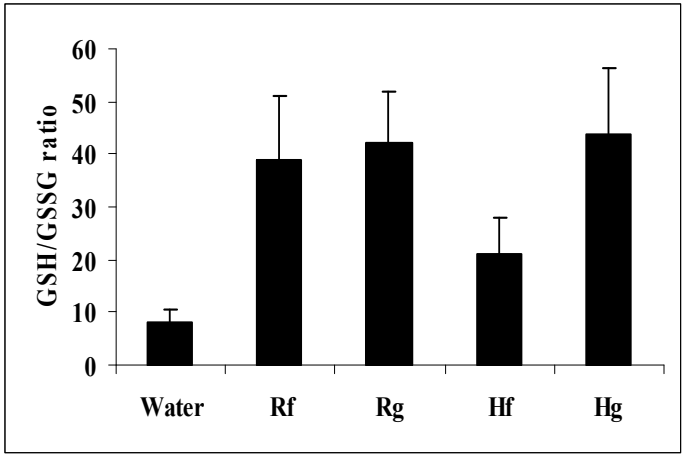

Figure 1 Effect of fermented and green rooibos (Rf, Rg) and honeybush (Hf, Hg) on the hepatic redox status (reduced to oxidized glutathione ratio) of rats consuming the various herbal teas for 10 weeks. The control group consumed water (Data are from reference 50)

Rooibos and honeybush also enhanced the activities of the phase II cytosolic enzyme, glutathione S-transferase alpha, while the green rooibos and honeybush teas also increased the activity of the phase II microsomal enzyme UDP-glucuronosyl transferase. Modulation of the oxidative status and phase II enzyme activity were suggested to be important events in the protection against adverse effects related to oxidative damage. Similarly, two other studies also reported on the hepatoprotective effect of traditional rooibos in rats that have been exposed to a potent liver pro-oxidant, carbon tetrachloride (CCl_4), with rooibos preventing the induction of lipidperoxidation by CCl_4 (*51*) and the prevention of oxidative stress in streptozotocin-induced diabetic rats with rooibos being recommended as an adjuvant support for the prevention and therapy of diabetic vascular complications (*52*). When considering the inhibition of lipidperoxidation, a few studies have reported on the activity of both traditional and green rooibos to inhibit/decrease such oxidative damage (*54,55*) *in vitro* as well as *in vivo* (52).

When comparing the antioxidant potencies of both the traditional/fermented and green/unfermented rooibos and honeybush, evidence is difficult to select for the best one, since the activity is highly dependent on the assay used, the nature of the raw material, and type of samples (aqueous extracts, enriched-extracts or solvent extracts) used**.**

Chemopreventive/Antimutagenic Properties

Initial studies demonstrated the *in vitro* antimutagenic properties of both traditional and green rooibos and honeybush extracts using various test systems. These systems included the *Salmonella* mutagenicity assay (*56*), COMET assay (*57*), chromosomal aberration assay using Chinese hamster ovary cells (*35*) and X-ray-induced oncogenic transformation using mouse embryo fibroblast cells (*36*). In general, all these studies reported positively on the *in vitro* protection/modulation of genetic damage/cell proliferation by various extracts of rooibos.

Subsequently, the *in vitro* antimutagenic findings have also been substantiated using experimental animal models. One of these studies conducted included a 10 week rooibos feeding in male Fischer rats (*58*) to evaluate possible *ex vivo* antimutagenic activity of brewed traditional and green rooibos and honeybush herbal teas. Novel findings from this study included that hepatic cytosolic fractions from rats consuming the green rooibos brew for 10 weeks significantly protected against 2-acetylaminofluorene (2-AAF)–induced mutagenesis in the *Salmonella* mutagencity assay with tester strain TA 98, using Aroclor 1254-induced microsomes for mutagenic activation, while green honeybush showed a marginal protective effect. When using aflatoxin B_1 (AFB_1) as mutagen both the traditional and green rooibos and honeybush significantly protected against the induced mutagenicity. The activation potential of hepatic microsomal preparations from rats consuming the rooibos teas were also evaluated and both rooibos teas and green honeybush reduced the activation of AFB_1 but not that of 2-AAF. Presumably, the rooibos and honeybush modulate different isoforms of the phase I drug metabolizing enzyme, cytochrome P450 directing the metabolism away from the formation of the putative mutagenic metabolite (*58*).

The cancer modulating properties of rooibos and honeybush were subsequently reported on and these studies provided the first evidence for the *in vivo* modulation of tumor promotion (*54,59,60*). In a 7,12-dimethylbenz[a]anthrazene-initiated, 12-O-tetra-decanoylphorbol-13-acetate promoted two-stage skin carcinogenesis model, methanolic fractions from traditional and green rooibos and honyebush were topically applied to the skin of ICR mice and significantly reduced the mean number as well as the size of tumors on these mice (*54*). In the skin tumor development model, the honeybush extracts "performed better" than the rooibos, as the honeybush extracts showing the highest inhibition (90% and 84.2% for the green and fermented extracts) (Fig. 2). This again suggests that one health beverage should not be artificially described as "better" than another based on any single test or *in vitro* antioxidant activity alone.

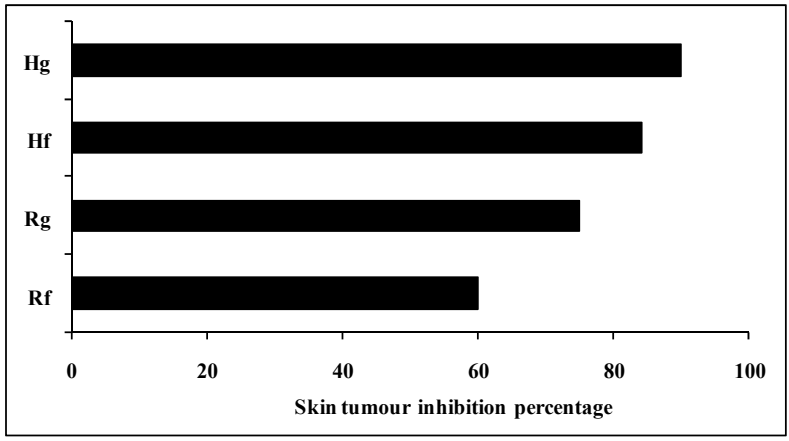

Figure 2 Inhibitory effect of topical application of rooibos and honeybush extracts on skin tumor development over a 20 week period in a two-stage mouse skin carcinogenesis model (Data are from reference 54)

The type of phytochemicals, their ratios and combination in addition to the specific assay systems chosen play a major role in the outcome. A recent study investigated the *in vitro* transport of aspalathin, the main rooibos polyphenol, as well as a green rooibos extract across the skin and intestinal epithelium (*61*). Only 0.07% and 0.08% of the initial aspalathin dose for the green rooibos extract and pure aspalathin solution penetrated the different layers of the skin, while 100% and 79% transported across Caco-2 cell monolayers, suggesting that the presence of other phytochemicals in the green rooibos extract may assist higher transport. When the cancer modulating properties of hot water rooibos and honeybush extracts were monitored in a liver carcinogenesis model it was the green rooibos that showed protection against fumonisin B_1-induced (FB_1) cancer promotion (*59*), while the other herbal extracts decreased either the smaller or larger sized foci, but not the total number of foci in the liver. Diethylnitrosamine (DEN) was used as cancer initiator, while the number and size of pre-neoplastic lesions staining positive for the placental form of gamma glutamyl transferase (GSTP) were scored. Green rooibos significantly reduced the number of pre-neoplastic foci in the liver (Fig. 3), presumably by arresting their growth (*59*). Recently a similar effect was reported using a site specific carcinogen, N-methylbenzylnitrosamine to induce esophageal cancer in male Fischer rats. Rats consumed a hot water extract of traditional and green rooibos for 25 weeks where after the number and size of esophageal papillomas were scored. Green rooibos and honeybush significantly inhibited the development of larger (>10 and <20 mm^3) papillomas as well as reduced the number and size of papillomas (*60*). Results from these studies could proof important in the development of cancer prevention strategies.

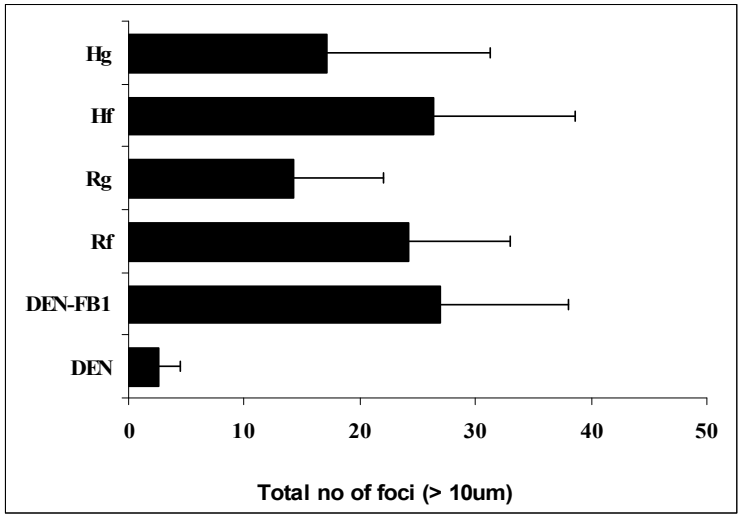

Figure 3 Effect of green and fermented rooibos (Rg, Rf) and honeybush (Hg, Hf) on the induction of GSTP$^+$ foci by combined treatment of DEN and FB_1 (Data from reference 59)

Other Bioactive Properties

Extracts of both rooibos and honeybush species have been shown to possess estrogenic activity *in vitro* (*62-65*). Some of these studies reported on the binding of certain phenolic compounds such as phyto-estrogens present in rooibos and honeybush, to the estrogen receptor using various cell lines. These results suggest intra and inter *Cyclopia* species variability with regards to estrogenic potency exists. Using experimental animal and *in vitro* models an aqueous extract of presumably fermented rooibos (15% w/v) produced a dose-dependent decrease in the mean arterial blood pressure of rats, as well as cause an antispasmodic effect mediated predominantly through K_{ATP} channel activation (*66*). This study also reported that the aqueous rooibos extract also possesses smooth muscle relaxing effect. The selective bronchodilatory effect of the tea extract was shared by one of its known flavonoid compounds, chrysoeriol, while orientin was found selective for its inhibitory effect on the gut. The investigators suggested that these observations may explain the medicinal use of rooibos tea in hyperactive gastrointestinal, respiratory and cardiovascular diseases with the potential to be developed as a remedy for the congestive airway disorders (*66*). As impaired immune responses is known to be the cause of many allergies, the effect of rooibos on the immune response has been examined in many cell cultures or experimental animal studies and showed that rooibos increased antibody responses and improved cell survival through the stimulation of interleukin-2 (IL-2) in splenocytes primed with ovalbumin (OVA) and CD3 (*67*). Recently the same group also reported that an aqueous rooibos fraction (mainly consisting of oligosaccharides and polysaccharides) increased immunoglobulin M production in anti-OVA-stimulated murine

splenocytes, associated with the production of IL-10. A comment was made that this extract could be of clinical use (*68*). Antimicrobial activity of rooibos and honeybush extracts has been reported in a few publications. Aqueous extracts of both fermented and green rooibos were shown to inhibit the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans*, *Saccharomyces cerevisea* and *Escherichia coli*, with the fermented extracts being more effective (*69*). In the case of *E. coli* the author suggested a bacteriostatic mechanism to be involved in the growth inhibition when using liquid cultures (*69*). Although these results could not be confirmed when an aqueous extract of rooibos was tested against *E. coli* using a solid medium zone inhibition method, antimicrobial activity against *Bacillus cereus*, *Micrococcus luteus* and *Candida albicans* were shown. (*70*). Recently extracts of green rooibos and honeybush (*C. subternata*, *C. genistoides*) showed growth inhibitory effects against *E. coli* using liquid cultures, with green rooibos and green *C. subternata* (100 mg/mL) also reducing spore germination of the plant pathogen, *Botrytis cinerea* (*71*). Alkaline extracts (1% sodium carbonate) of fermented rooibos (containing an acidic polysaccharide) were reported to suppress the cytopathic effects of HIV *in vitro* (*72*) using infected MT-4 cells. It was suggested by the authors that the extracts possibly inhibited the virus to bind to the CD4 receptor extracts from rooibos tea.

Pharmacocnosy

Few human studies or clinical trials examining the potential health and/or therapeutic applications of rooibos have been reported. During the early 1980s, a small (n=7) study was conducted in humans suffering from asthma or hay fever. Neither the ingestion of traditional rooibos nor the topical application of a rooibos poultice exhibited any antihistaminic effects (*73*). Nearly a decade later, another human study was published that showed a positive effect an infusion of rooibos had on patients with atopic dermatitis and *herpes simplex* viral infection (*74*). A decreased itching and induced-inflammation was reported as well as a decreased incidence of *herpes simplex*. As yet, no human studies on immune responses have been published.

Another small (n=10) human study in the late 1970s reported on the effect of rooibos on iron absorption (*32*). No detrimental effect on iron absorption was shown after the subjects had consumed traditional/fermented rooibos (200 mL containing milk and sugar) when compared with the control group consuming water. Subsequently, a more recent study published in 2005 confirmed these results that the intake of 200 mL of traditional rooibos (with milk and sugar) per day for 16 weeks by school children did not have any adverse effects on their iron status (*75*).

An eight week randomized placebo-controlled intervention study reported on the antioxidant status of lead factory workers drinking traditional/fermented rooibos (*76*). The modulation of oxidative status of these workers was shown by a decreased level of lipid peroxidation (measured as malondialdehyde in the plasma) and increased level of blood glutathione (GSH). The authors suggested that rooibos could play a beneficial preventive role in occupationally exposed

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workers. Although not reporting on the consumption of an aqueous brew of traditional rooibos, another study was conducted using an aspalathin-enriched extract of green rooibos (*77*). Subjects were given such a tablet (containing 15% aspalathin) twice daily for 2 weeks where after various blood parameters including antioxidant status biomarkers were monitored. No changes could be seen except for a minor decrease in the antioxidant status of these subjects when using a xanthine/xanthine oxidase test system (*77*).

Only recently the first human intervention study was completed in the Western Cape Province of South Africa to monitor the modulation of oxidative stress by traditional rooibos in adults at risk for developing heart disease (http://sarooibos.co.za/content/view). This study also served as the first scientific proof for human safety when considering the various clinical pathology results (Table IV).

Table IV. Effect of Traditional Rooibos Consumption on Selected Blood Clinical Pathology Markers Related to Liver and Kidney Function.

Analyte	Baseline	Wash out	Rooibos	Control
AST (U/L)	24.5 ± 10.8	21.1 ± 7.2	24.1 ± 14.2	17.3 ± 7.3
ALT (U/L)	26.4 ± 15.9	23.3 ± 12.5	21.4 ± 14.0	15.0 ± 8.8
GGT (U/L)	32.4 ± 21.2	31.5 ± 15.9	30.6 ± 17.6	27.9 ± 15.1
ALP (U/L)	81.6 ± 35.5	85.5 ± 27.8	79.0 ± 25.0	64.4 ± 22.6
LDH (U/L)	155.0 ± 51.8	150.7 ± 31.7	179.9 ± 44.2	182.8 ± 68.5
Total protein (g/L)	76.2 ± 31.3	80.4 ± 22.2	66.6 ± 4.4	65.9 ± 18.5
Urea (mmol/L)	5.3 ± 1.6	5.3 ± 1.7	4.1 ± 1.1	4.3 ± 1.3
Creatinine (µmol/L)	98.4 ± 45.5	93.5 ± 22.5	78.0 ± 19.1	68.5 ± 28.0
D Bilirubin (µmol/L)	3.5 ± 1.8	3.0 ± 1.5	3.6 ± 2.1	8.2 ± 11.2
T Bilirubin (µmol/L)	10.1 ± 3.8	10.3 ± 5.9	9.9 ± 3.9	15.6 ± 12.0
Glucose(mmol/L)	5.4 ± 1.3	5.9 ± 2.7	4.8 ± 0.6	5.5 ± 1.7
Total iron (µmol/L)	16.0 ± 5.4	17.1 ± 4.7	15.7 ± 4.8	13.9 ± 3.9

NOTE: All samples (n=40) were done in triplicate and values in columns represent means ± SD. Abbreviations: aspartate aminotransferase (AST), alanine aminotransferase (ALT); gamma glutamyl transferase (GGT); Alkaline phosphatase (ALP); lactate dehydrogenase (LDH); Conjugated bilirubin (D bilirubin); total bilirubin (T bilirubin).
SOURCE: Unpublished data (Dr JL Marnewick, Oxidative Stress Research Centre, Cape Peninsula University of Technology, Bellville, South Africa).

Forty adults (male and female) between the ages of 30 and 65 were required to consume 6 cups of traditional rooibos daily for 6 weeks. No adverse effects were reported by any of the participants during the study period as also shown by the various liver (AST, ALT, GGT, ALP, LDH) and kidney (creatinine total protein, urea) parameters measured in the serum of the participants. Preliminary results obtained from this study indicated that the consumption of rooibos protected the body against oxidative damage as shown by a decrease in two lipid peroxidation biomarkers, conjugated dienes, a primary oxidation product, as well as malondialdehyde, a secondary oxidation product. An increase in the redox status of the participants as seen by an increase in circulating GSH levels, decrease in GSSG levels with a resultant increase in GSH/GSSG ratio was also reported (unpublished data, Dr JL Marnewick, Oxidative Stress research Centre, Cape Peninsula University of Technology, South Africa). When compared to the control (water) phase, the levels of circulating conjugated dienes after completion of the rooibos intervention phase decreased by nearly 35%. When considering MDA, the levels of TBARS decreased with 52% after completion of the rooibos intervention when compared to the control (water intervention) phase. Lipid peroxidation is associated with cellular injury and that the byproducts are present at increased levels in oxidative damage. This study thus suggested that rooibos protects the body against oxidative damage which is in line with the findings of the study by Nikolova and co-workers (*76*). Results from this study will be finalized and a paper will be submitted to a medical journal for publication. Further evidence from human studies is required before

strong conclusions can be made regarding the association between rooibos and heart disease. No published reports describing the effects of honeybush in humans have appeared in any English peer-reviewed journals to date.

Conclusions

Humans are constantly seeking to advance their health and alleviate various ailments with herbal remedies. In this review, which reflects an integrated approach reporting on different *in vitro*, *ex vivo*, experimental animal test systems and few human clinical studies the roles rooibos and honeybush can play in the improvement of human health was discussed. Results from the body of evidence relative to the chemistry, biochemistry, and biological activity coupled to initial clinical studies clearly suggest that both of these South African teas provide healthy and beneficial herbal teas which warrant further controlled clinical investigations into the potential health modulating properties of these two promising natural products.

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