REFERENCES

RED BUSH TEA
Aspalathus linearis
Borbonia pinifolia
Aspalathus contaminata

1. In the Lawrence Review of Natural Products (Aug 1990) we read that it is also known as rooibos tea. The plant is native to South Africa and is cultivated as a substitute for common tea. The leaves and twigs are collected, washed, bruised, fermented, dried, cut and packaged for use in preparing teas. During this procedure, the leaves change from green to brick red due to the release of a red pigment found in the leaves and stems.

It is almost totally lacking in physiologically active compounds. Consequently, red bush tea is selected as a fragrant, non-stimulating beverage. It contains no caffeine and is low in tannins (<5%). It contains a relatively high in vitamin C. It is not toxic. It is a mild and sedating tea.

2. In the Lawrence Review of Natural Products (May 1997)

Scientific names: Aspalathus linearis (Burm. f.) R. Dahlgr. This plant is also referred to as *Borbonia pinifolia* Marloth or *Aspalathus contaminata* (Thunb.) Druce. Family: Leguminosae.

Common names: Red bush tea, rooibos tea.

History: "Bush Teas" are common throughout Africa and are frequently used as substitues for common tea. Red Bush tea has been popular in South Africa for decades, and commercial preparations are sometimes found in Europe and the United States. One reason for the popularity of this tea is its almost total lack of physiologically active compounds. Consequently, red bush tea is selected as a fragrant, non-stimulating beverage.

Chemistry: Red bush tea and its *protective* and *suppressive* effects have been studied. Suppression of mutagenic activity of "certain potent mutagens" has been performed n mice. Oncogenic transformation of mouse cells induced by x-rays was suppressed in the presence of the tea extract. Suppression variability was dependent upon extract concentration, and length of treatment time. Prevention of age-related accumulation of lipid peroxides in rat brain areas have been additionally demonstrated. Flavonoids contained in the tea show antioxidative qualities both *in vitro* and *in vivo*. Red bush tea's radioprotective effects may be due to a "free radical scavenging" mechanism.

Toxicology: No reports have been identified regarding toxicity with this plant or its teas. A single article reports salmonella contamination from rooibos tea, possibly from lizard origin.

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Rooibos Tea: Research into Antioxidant and Antimutagenic Properties

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by Laurie Erickson

Antioxidants are hot topics in the health news these days, and an herbal tea called rooibos (pronounced ROY-boss) is becoming popular partly because it is being marketed as a healthy beverage with high levels of antioxidants. The rooibos plant (*Aspalathus linearis* (Burm. f.) Dahlgren, Fabaceae) is a South African flowering shrub used to make a mild-tasting tea that has no caffeine, very little tannin, and significant amounts of polyphenol antioxidants. Although the tea is new to many Americans, it has been made in the Cedarberg mountain region of South Africa for generations. Distributors are promoting the tea for numerous health benefits, citing recent studies that show some antioxidants found in rooibos tea may protect against cancer, heart disease, and stroke. What's the evidence for these claims?

A Note on Tea Terminology

In the strict sense, the word *tea* has been reserved for infusions made from leaves of the evergreen shrub *Camellia sinensis* (L.) Kuntze, Theaceae, while infusions made from herbs such as rooibos have been called *tisanes*. Over time, however, the common use of the word tea has been extended to include herbal infusions, and this relaxed usage is followed here. Rooibos is often referred to as red tea because it makes a vibrant red-colored tea, which can be confusing because black tea and hibiscus herbal tea are also sometimes called red tea.

Botanical Description

Rooibos is a shrubby legume that is indigenous to the mountains of South Africa's Western Cape.[1-3] The genus *Aspalathus* includes more than 200 species native to South Africa.[2-5] *A. linearis* is a polymorphic species; various wild forms have been described, each with characteristic morphology and geographical distribution.[1-3] Some forms are prostrate and remain less than 30 cm (1 foot) tall, while other forms grow erect and may reach up to 2 m (about 6 feet) in height.[1-3,6] The types of wild rooibos that have been used to make tea are sometimes referred to as the Red, Black, Grey, and Red-Brown types.[1,2]

The type of *A. linearis* that is cultivated commercially for tea is the Red type, also known as the Rocklands type;[1,6] it is native to the Pakhuis Pass area in the northern Cedarberg region.[6] The Rocklands type grows erect, up to 1.5 m (about 5 feet) in height. It has a single basal stem that divides just above the ground surface into multiple thin branches that carry bright green, needle-like leaves of about 10—40 mm (0.4—1.6 inches) in length.[7] The plant

produces small yellow flowers in spring through early summer,[6] and each flower generates a one-seeded leguminous fruit.[4,5]

Rooibos has adapted to coarse, nutrient-poor, acidic soil and hot, dry summers.[4,5,8] In addition to a network of roots just below the soil surface, the plant has a long tap root that reaches as deep as 2 m (about 6 feet) and helps the plant find moisture during summer drought.[5] As a legume, rooibos contains nodules of nitrogen-fixing bacteria on its roots; this characteristic helps the plant survive in the poor Cedarberg soils and minimizes the need for fertilizing commercial crops with nitrogen.[8] The bacteria convert nitrogen dioxide to biologically useful ammonia in a process known as *nitrogen fixation*. The plant absorbs the nitrogen and benefits from it in exchange for providing the bacteria with food sources created from photosynthesis.

One study found genetic variations between four morphologically different populations of *A. linearis*.[1] The authors suggest that the wild forms of *A. linearis* might be used to improve characteristics, such as yield and disease resistance, of the cultivated form. They also observe that because the cultivated Rocklands form is being grown outside of its original Pakhuis Pass location, this introduction of the cultivated form into new areas could threaten the genetic integrity of the wild forms in these areas.

A later study[7] showed genetic differences between populations of *A. linearis* that are *sprouters* (plants that can resprout from a deep rootstock to regenerate after a fire) and populations that are *seeders* (plants that rely on producing plentiful seeds to reproduce). The authors suggest that reseeding is the primitive character state in *A. linearis* and resprouting is a derived state that evolved to help the plant survive in a region prone to wildfires.

Rooibos growing in the Cedarberg region of South Africa. Photo ©2003 Rooibos Ltd./SunnRooibos

The rooibos plant that is commercially grown for tea is the seeder type.[7]

In addition to differences in morphology and genetics, researchers have found differences in chemistry between various populations of *A. linearis*.[6,9] Van Wyk, of the Department of Botany at Rand Afrikaans University, presented results of his tests on the different wild populations of rooibos, showing significant variations in the polyphenol profile by population.[9]

Historical Background

More than 300 years ago, indigenous inhabitants of the mountainous regions of South Africa's Western Cape were the first to collect wild rooibos and use it to make tea.[10] These people discovered that they could brew a sweet, tasty tea from rooibos leaves and stems that they cut, bruised with wooden hammers, fermented in heaps, and then sun-dried. Botanists first recorded rooibos plants in 1772 when they were introduced to the tea by the Khoi people.[10]

Rooibos became a cultivated crop by the early 1930s, has been grown commercially since World War II, and now is exported to countries worldwide, including Germany, Japan, the Netherlands, England, Malaysia, South Korea, Poland, China, and the United States.[10] In 1999, about 29 percent of South Africa's total rooibos sales were exported to 31 countries.[10] The quantity of rooibos exported in 2000 was two and a half times greater than the quantity exported in 1999, and exports continue to grow.[10] The small towns of Clanwilliam and Wupperthal, north of Cape Town in the Cedarberg region, have a long history of rooibos cultivation; these towns are popular tourist stops because of their beautiful rural scenery and their role in the rooibos industry.

Roughly 70 percent of the bulk rooibos that is exported goes through Clanwilliam-based Rooibos Ltd. <www.rooibosltd.co.za>, a partnership of private growers/processors and a cooperative of large and small farmers in the area. The rooibos is sold in a variety of products in Europe, Asia, and, increasingly, America. Other South African companies that market rooibos tea products include Khoisan, Cape Natural Tea Products, and Coetzee & Coetzee. International demand for rooibos has been increasing since trade sanctions against South Africa were lifted following the demise of apartheid in the 1990s. Since 1999, the nonprofit organization Agribusiness in Sustainable Natural African Plant Products (ASNAPP, <www.asnapp.org>) has helped small farmers in and around Wupperthal to introduce sustainable methods of rooibos cultivation that allow them to compete in the world market. ASNAPP is sponsored by the U.S. Agency for International Development, Rutgers University, and Stellenbosch University. Through Stellenbosch University, ASNAPP also helped the farmers at Wupperthal fund construction of a tea court to process rooibos.

Rutgers University provides a quality control program for ASNAPP's Wupperthal tea program, evaluating parameters such as color, taste, aroma, pH, moisture content, cleanliness, total phenol content, and antioxidant capability for tea samples collected from the industry in general and from all the growers in the Wupperthal tea program.[11] Data from their analyses are made available to the farmers and also to prospective buyers via product specification sheets.

The Perishable Products Export Control Board (PPECB) of South Africa ensures that all exported rooibos products pass a phytosanitary inspection and are certified to be free of bacteria and impurities.[4,10] In order to pass these health and safety tests, rooibos producers steam pasteurize the tea as the final step before packing. Organic rooibos is also monitored by

various international organizations that provide organic certification, such as the German firms Ecocert and Lacon.

Harvesting and Processing: Fermented and Unfermented Rooibos

When rooibos is cultivated commercially, the needle-like leaves and stems are usually harvested in the summer, which corresponds to January through March in South Africa.[4] The plants are cut to about 30 cm (1 foot) from the ground at harvest time and begin another major growth cycle the following spring. The harvested rooibos is processed two different ways, producing two types of tea. The green leaves and stems are either bruised and fermented or immediately dried to prevent oxidation. The traditional fermented tea is processed today in much the same way as the indigenous people processed it hundreds of years ago, including the sun-drying step, but the tools are more sophisticated now.

Rooibos grows in the Cedarberg Mountain region of South Africa.

The fermented type is called red tea because fermentation turns the leaves and the resulting tea a rich orange/red color; this distinctive color led to the Afrikaans name *rooibos*, which means "red bush." The unfermented type, often called green rooibos, contains higher levels of polyphenol antioxidants because fermented rooibos loses some antioxidants during the fermentation process. The unfermented type was developed to maximize antioxidant levels in response to recent interest in the health benefits associated with the antioxidants found in *C. sinensis* teas. Unfermented rooibos tea is a tan/yellow color rather than the rich reddish color of fermented rooibos.

Both types of rooibos tea are available plain or flavored, loose or in tea bags, organic or conventionally grown. Rooibos is graded according to color, flavor, and cut length, with the highest grade labeled "supergrade." The tea has a smooth, non-bitter flavor that is pleasant hot or chilled. The unfermented variety has a very mild "green" taste reminiscent of green tea but without the astringency; the fermented type is quite different, with a stronger sweet and fruity taste. The mild flavor of rooibos has made it popular in multi-ingredient herbal tea blends.

Antioxidants in Rooibos

Free radicals (unstable molecules that have lost an electron) can damage the DNA in cells, leading to cancer, and they can oxidize cholesterol, leading to clogged blood vessels, heart attack, and stroke. Antioxidants can bind to free radicals before the free radicals cause harm. Some antioxidants are called polyphenols because these substances contain a phenolic ring in their chemical structure. Polyphenols are common in plants; they act as pigments and sunscreens, as insect attractants and repellants, and as antimicrobials and antioxidants.[12,13]

The polyphenol group is further divided into subgroups such as flavonoids and phenolic acids. Polyphenols can also be classified as *monomeric* (molecules containing a single unit) or polymeric (larger molecules containing more than one unit). As described in this section, laboratory studies have found that rooibos tea contains polyphenol antioxidants, including flavonoids and phenolic acids, that are potent free radical scavengers.

Flavonoids: The polyphenol antioxidants identified in rooibos tea include the monomeric flavonoids aspalathin, nothofagin, quercetin, rutin, isoquercitrin, orientin, isoorientin, luteolin, vitexin, isovitexin, and chrysoeriol.[14-19] Currently, rooibos is the only known natural source of aspalathin.[15] Nothofagin is similar in structure to aspalathin and has only been identified in one other natural source besides rooibos: the heartwood of the red beech tree (*Nothofagus fusca* (Hook F.) Oerst, Nothofagaceae), which is native to New Zealand.[20]

A recent analysis of fermented rooibos measured the levels of all the flavonoids listed above except nothofagin (see Table 1).[19] Of the 10 flavonoids measured, the three that occurred in largest amounts were aspalathin, rutin, and orientin, followed by isoorientin and isoquercitrin. Nothofagin was identified by mass spectrometry but was not quantified because a standard was not available. The amount of nothofagin in fermented and unfermented rooibos was estimated to be about three times less than aspalathin in one study.[20] Aspalathin and nothofagin arepresent in relatively large amounts in unfermented rooibos tea,[19,20] but some of the aspalathin and nothofagin oxidizes to other substancesduring fermentation; thus, fermented rooibos contains less aspalathin and nothofagin than unfermented rooibos.[20] The change in polyphenol composition is the reason the tea changes color with fermentation.[20]

Table 1: Flavonoids in Aqueous Extract of Fermented Rooibos (mg/g +/- SD)

Flavanoid	(mg/g +/- SD)
isoorientin	0.833 +/- 0.007
orientin	1.003 +/- 0.010
aspalathin	1.234 +/- 0.010
vitexin	0.330 +/- 0.002
rutin	1.269 +/- 0.006
isovitexin	0.265 +/- 0.002
isoquercitrin and hyperoside	0.429 +/- 0.002
luteolin	0.029 +/- 0.001
quercetin	0.107 +/- 0.002
chrysoeriol	0.022 +/- 0.001
total	5.521 +/- 0.003

Note: The extracts were prepared using 1 g of rooibos in 60 ml of hot distilled water, steeped for 10 minutes. After removal of the tea leaves, the solution was cooled and filtered. The table gives the amounts of flavonoids in mg per g of extract.

Source: Bramati L, Minoggio M, Gardana C, Simonetti P, Mauri P, Pietta P. Quantitative characterization of flavanoid compounds in Rooibos tea (Aspalathus linearis) by LC-UV/DAD. *J Agric Food Chem* 2002 Sep 25;50(20):5513-9.

Phenolic Acids: In addition to flavonoid antioxidants, rooibos also contains phenolic acids that have been shown to have antioxidant activity.[14,18,21] Like flavonoids, phenolic acids are polyphenol substances that are found in fruits, vegetables, and whole grains. The phenolic acids identified in rooibos tea, in decreasing order of antioxidant activity as measured in one study[21] with the commonly used 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, include caffeic acid, protocatechuic acid, syringic acid, ferulic acid, vanillic acid, *p*-hydroxybenzoic acid, and *p*-coumaric acid.[14,18] Using the DPPH assay, caffeic acid was just as active an antioxidant as the most potent flavonoids tested (quercetin, isoquercitrin, and aspalathin).[21]

Total Polyphenol Content: Despite some promotional claims, a serving of rooibos tea has less total polyphenols than the same size serving of green or black tea. Serving size varies, but for comparison purposes a 150 to 200 ml serving is often used (about 3/4 of a standard baking measuring cup). Elizabeth Joubert, Ph.D., specialist researcher at South Africa's ARC Infruitec-Nietvoorbij and a rooibos expert, says that the total polyphenol content of an average 150 to 200 ml serving of rooibos tea can be as much as 60 to 80 mg, depending on factors such as the brewing time and amount of leaves used.[22] For comparison, one study found that brewing black tea leaves for 1 to 3 minutes at a concentration of 1 g leaves per 100 ml water resulted in black tea that contains 128 to 199 mg of polyphenols per 200 ml serving of tea.[23] The types of polyphenols in rooibos tea are different than those in green and black teas, so the potential health benefits of the teas cannot be compared solely on their total polyphenol content. Rooibos tea does not contain epigallocatechin gallate (EGCG), which is a polyphenol in green tea that has shown anticarcinogenic and antioxidant capabilities, but many of the polyphenols in rooibos tea are also strong antioxidants.

Quercetin and Luteolin: Two of the flavonoids in rooibos tea, quercetin and luteolin, are potent antioxidants found in many fruits and vegetables. Studies *in vitro* (in the test tube) have shown that these antioxidants can cause cancer cells to "commit suicide," referred to as apoptosis.[24-27] Quercetin decreased primary tumor growth and prevented metastasis in a model of pancreatic cancer.[25] Luteolin and quercetin inhibited proliferation of thyroid[28] and colon[29] cancer cells, respectively, *in vitro*. Quercetin inhibited cyclooxygenase-2 (COX-2) expression in colon cancer cells, which may help prevent colon cancer.[30,31] Both luteolin and quercetin can block the formation of lipid peroxides.[32-34]

Although studies like these show quercetin and luteolin are strong antioxidants, researchers haven't yet determined whether enough of either of these two flavonoids are present in rooibos tea and absorbed by the body to have beneficial effects. As shown in Table 1, recent analysis of fermented rooibos found considerably more quercetin than luteolin,[19] but even quercetin was present in much lower amounts than aspalathin, orientin, and rutin.

Based on the data in Table 1, a 150 ml serving of fermented rooibos tea made with 2.5 g of tea leaves has about 0.27 mg of quercetin; for comparison, one study found that *C. sinensis* contains 1.5 to 3.75 mg of quercetin per 150 ml serving of tea.[35] A previous study[36] found 1.5 mg of quercetin per 150 ml serving of fermented rooibos, but that may be an upper limit. Joubert says that the 1.5 mg estimate is probably high,[22] but emphasizes that these estimates will vary with parameters such as the brewing time and the amount of water and tea leaves used. At any rate, the amount of quercetin per serving of rooibos is a small percentage of the total polyphenol content per serving of rooibos.

Aspalathin and Nothofagin: A unique polyphenol that is one of the most abundant monomeric flavonoids in rooibos tea,[19,20] aspalathin seems to contribute to the antioxidant capabilities of rooibos,[21] but aspalathin is not as well studied as quercetin and luteolin. Nothofagin is similar in structure to aspalathin and may have similar antioxidant capabilities.

Joubert says that chief research technologist Petra Snijman of the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC) at the Medical Research Council of South Africa recently developed a way to isolate pure aspalathin and nothofagin from rooibos. Joubert says, "According to unpublished *in vitro* studies done at ARC Infruitec-Nietvoorbij, aspalathin compared well with quercetin in terms of antioxidant activity, except in a fat medium where quercetin demonstrated much higher potency than aspalathin. What is important in these comparative studies is the test environment. Relative efficacy will depend

on the test system used (the polarity of the medium, the type of free radical that needs to be scavenged, etc.)."[22]

Joubert co-authored a study[21] that found aspalathin compared well to other antioxidants with the DPPH radical scavenging assay. The study measured the antioxidant capability of many of the flavonoids and phenolic acids found in rooibos tea and compared them to several reference standards such as alpha-tocopherol (vitamin E). The percent inhibition of the DPPH radical by quercetin, isoquercitrin, aspalathin, rutin, luteolin, and alpha-tocopherol was 98.27, 91.99, 91.74, 91.18, 90.85, and 75.10, respectively (using a 0.25 mole ratio of antioxidant to DPPH). All of the flavonoids tested showed potent hydrogen donating abilities with DPPH except for vitexin, which only had a 7.26 percent inhibition even at a 0.5 mole ratio to DPPH.

According to the data in Table 1, a 150 ml serving of fermented rooibos made with 2.5 g of tea leaves has about 3 mg of aspalathin; since the amount of nothofagin was measured to be three times less than aspalathin in one study,[20] a 150 ml serving of fermented rooibos has on the order of 1 mg of nothofagin. A serving of unfermented rooibos has considerably more aspalathin and nothofagin than an equal serving of fermented rooibos because a portion of these flavonoids oxidizes to other substances during fermentation.[20]

Orientin and Rutin: Orientin and rutin are two of the other most abundant monomeric flavonoids in rooibos,[19] and both have been associated with health benefits. Orientin is a potent free radical scavenger. It reduced by half the number of cancer-associated changes in cells of human blood exposed to radiation.[38] When mice were exposed to radiation, orientin protected against lipid peroxidation in the liver and also reduced damage to the bone marrow and gastrointestinal tract.[39,40] Rutin, a flavonoid found in buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) and some fruits and vegetables, seems to help maintain the strength of capillary walls; oral rutin as well as oral and topical o-(beta-Hydroxylethyl)-rutoside (HR) have been used to treat hemorrhoids, varicose veins, and the lower leg edema associated with venous insufficiency and venous hypertension.[41-46] According to the data in Table 1, a 150 ml serving of fermented rooibos tea made with 2.5 g of tea leaves has about 2.5 mg of orientin and 3.2 mg of rutin.

Total Antioxidant Capability: Although the 10 flavonoids in Table 1 are important because they are known to have antioxidant properties, they only represent a small percentage of the total polyphenol content of a serving of fermented rooibos tea. A 150 to 200 ml serving of rooibos can have up to 60 to 80 mg of total polyphenols,[22] and Table 1 shows that a 150 ml serving of fermented rooibos made with 2.5 g of leaves has about 14 mg of the 10 flavonoids in the table. Many other polyphenols are present, but they have not all been identified or quantified.

To assess the antioxidant capability of rooibos tea as a whole, researchers compared the antioxidant activity of rooibos tea extracts to that of green and black tea extracts with the DPPH radical scavenging assay as well as the beta-carotene bleaching method.[47] All the teas showed strong antioxidant activity with both methods. Using the DPPH method, the ranking from highest to lowest antioxidant activity was green tea (90.8 percent inhibition), unfermented rooibos (86.6 percent), fermented rooibos (83.4 percent), and black tea (81.7 percent). Green tea was significantly higher than the others ($P < 0.05$), but the other three teas did not differ from each other significantly with respect to DPPH inhibition. Using the beta-carotene bleaching method, the ranking was green tea, black tea, fermented rooibos, and unfermented rooibos. The relative ranking varies with the type of test because the substance to be tested will have different reactivity to the different oxidizing agents used. These tests

only measure the antioxidant capability of substances outside of the body and don't provide data on whether the antioxidants are absorbed by the body and effective after the food is consumed.

In this study, all the tea extracts were diluted to the same amount of soluble solids rather than to the amounts of solids found in the teas.[47] This method allows a comparison of antioxidant capability on a mass equivalent basis, but does not reflect a comparison of the antioxidant strength of equal volume servings of the teas. Although the soluble solid content varies with the method of tea preparation, it usually decreases in the order green tea, black tea, unfermented rooibos, fermented rooibos.[47] The percent of soluble solids represented by polyphenols is similar for the four teas and the DPPH antioxidant activity is similar on a mass equivalent basis, so the DPPH antioxidant capability of equal-sized servings will decrease in the order of the soluble solid content.[47] Black and green teas have over twice as much soluble solids as rooibos tea when prepared conventionally, so over two 200 ml servings of rooibos tea would need to be consumed to receive the same antioxidant benefit (as measured by DPPH) as one 200 ml serving of black or green tea (or the rooibos would need to be brewed to twice the standard concentration).[47] This result agrees with the data given previously for 60 to 80 mg polyphenols for a 150 to 200 ml serving of rooibos tea[22] as compared to 128 to 199 mg polyphenols for a 200 ml serving of black tea.[23]

Table 3: Percent Aberrations in Cells Treated With B(a)P or MMC and Post-Treated With Tea Extract +/- S9

Mutagen	Tea Extract (microgram/ml)		without S9 (%)	with S9 (%)
B(a)P	None		44	35
	Green tea	25 or 125	40	28
		50 or 250	45	19 *
		100 or 500	48	19 *
		200 or 1000	42	20 *
	Rooibos	25 or 125	35	23
		50 or 250	34	19 *
		100 or 500	27 *	17 **
		200 or 1000	22 ***	11 ***
MMC	None	42	44	
	Green tea	25 or 125	49	48
		50 or 250	48	43
		100 or 500	40	33
		200 or 1000	43	27 *
	Rooibos	25 or 125	45	48
		50 or 250	36	38
		100 or 500	22 **	29 *
		200 or 1000	22 **	23 **

Note: Tea extracts were prepared with 50 g of tea leaves and 1.5 l of boiling water. Cells were treated with 100 microM B(a)P and S9 for 3 hours or 1 microM MMC (no S9) for 1 hour, and then cells were post-treated with tea extracts for 3 hours with S9 or for 20 hours without S9. The larger concentrations of tea extracts were used with S9. Significant difference: *$0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$.

Key: S9 = rat liver enzyme; B(a)P = benzo[a]pyrene; MMC = mytomycin C

Source: Sasaki YF, Yamada H, Shimoi K, Kator K, Kinae N. The clastogen-suppressing effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice. *Mutat Res* 1993;286(2):221-32.

The studies referenced above show that rooibos tea contains antioxidants that have positive effects when tested as isolated substances and that the tea as a whole has good antioxidant activity *in vitro*. So, do all these antioxidants in rooibos tea lead to health benefits for tea drinkers?

Rooibos Research in Live Animals and Animal Cells

Laboratory studies have demonstrated potential health benefits of rooibos *in vitro* (in test tubes) and *in vivo* (in live animals), but human studies have not been conducted. Much more research is needed, but the studies so far look intriguing.

Fermented Rooibos against Mutagens: Researchers found that fermented rooibos tea reduced cancer-associated changes in animal cells induced by the mutagens benzo[a]pyrene (B(a)P) and mitomycin C (MMC) both *in vitro* and *in vivo*.[48] The *in vitro* part of the study measured chromosomal aberrations in animal cells caused by exposure to the mutagens. The cells were treated with tea extract either at the same time as the mutagen or after the mutagen. Some of the tests used rat liver microsomal enzyme, called S9, to provide metabolic activation of the mutagen; B(a)P requires metabolic activation, but MMC can act with or without it.

Both green tea and rooibos tea suppressed aberrant cells caused by B(a)P and MMC in the presence of S9, but rooibos showed a greater suppression of aberrant cells than did green tea (see Table 2). In fact, when the cells were exposed to B(a)P and S9 simultaneously with rooibos tea, the highest concentration of rooibos tea (1000 microgram/ml) completely inhibited the aberrant cells, bringing their percentage down to the level of the controls that were not exposed to any mutagen. Also, rooibos tea suppressed aberrant cells caused by MMC both with and without the presence of S9, but green tea showed no suppression without S9. Treating the cells simultaneously with the mutagen and tea extract caused a greater protective effect than treating the cells with tea extract following exposure to the mutagen (compare Tables 2 and 3).

Table 2: Percent Aberrations in Cells Treated Simultaneously with Tea Extract and the Mutagen B(a)P or MMC (+/- S9)

Mutagen	Tea Extract (microgram/ml)		without S9 (%)	with S9 (%)
None	None		3	4
B(a)P	None		NA	35
	Green tea	125	NA	25
		250	NA	20 *
		500	NA	19 *
		1000	NA	8 ***
	Rooibos	125	NA	9 ***
		250	NA	7 ***
		500	NA	6 ***
		1000	NA	3 ***
MMC	None		40	43
	Green tea	125	42	38
		250	41	35
		500	38	23 **
		1000	44	18 ***
	Rooibos	125	25 *	15 ***
		250	15 ***	10 ***
		500	9 ***	8 ***
		1000	8 ***	9 ***

Note: Tea extracts were prepared with 50 g of tea leaves and 1.5 l of boiling water. Cells were treated with 100 microM B(a)P and S9 for 3 hours or 1 microM MMC (+/- S9) for 1 hour. Significant difference: *0.01 < P < 0.05, ** 0.001 < P < 0.01, *** P < 0.001.
Key: S9 = rat liver enzyme; B(a)P = benzo[a]pyrene; MMC = mytomycin C
Source: Sasaki YF, Yamada H, Shimoi K, Kator K, Kinae N. The clastogen-suppressing effects of green tea, Po-lei tea and Rooibos tea in CHO cells and mice. *Mutat Res* 1993;286(2):221-32.

In the *in vivo* part of this study, mice were given oral doses of tea and an injection of B(a)P or MMC.[48] The researchers measured the frequency of micronucleated reticulocytes (MNRETs), which are cells with damaged DNA that may lead to cancer. In one experiment, a single oral dose of tea (1 ml of 0.2 percent green tea or 0.1 percent rooibos tea) was given 6 hours prior to an injection of MMC and the number of MNRETs was counted at 24, 48, and 72 hours after the MMC. Rooibos tea and green tea provided similar inhibition of the

frequency of MNRETs. After 48 hours, rooibos tea reduced the level of MNRETs by about 34 percent, and green tea reduced the level by about 38 percent. When the mice received the single dose of tea either after the mutagen or 24 hours prior to the mutagen, neither green tea nor rooibos tea reduced the frequency of MNRETs.

Table 4: Thiobarbituric Reactive Substances (TBARS) (nmol/g)

Area of Brain	24-month old no rooibos	24-month old with rooibos	5-week old no rooibos
frontal cortex	120	80	80
occipital cortex	115	70	80
hippocampus	80	40	50
cerebellum	115	80	85

Source: These data are approxmated from the bar chart in Figure 1 of Inanami O, Asanuma T, Inukai N, Jin T, Shimokawa S, Kasai N, Nakano M, Sato F, Kuwabara M. The suppression of age-related accumulation of lipid peroxides in rat brain by administration of Rooibos tea (*Aspalathus linearis*). *Neurosci Lett* 1995;196(1-2):85-8.

When the teas were given as one oral dose daily for 28 days and then the mutagen was injected on day 29, both rooibos tea and green tea reduced the frequency of MNRETs caused by B(a)P. Daily doses of 0.2 percent green tea reduced MNRETs by about 62 percent 48 hours after B(a)P exposure, and daily doses of 0.1 percent rooibos tea reduced MNRETs by about 49 percent. Daily doses of 0.1 percent rooibos tea reduced MNRETs by about 34 percent 48 hours after MMC exposure, but daily doses of green tea did not provide a significant reduction with MMC.

Fermented Rooibos against Irradiation: Another research group found that extract of fermented rooibos tea reduced cancerous transformation of mouse cells exposed to x-rays *in vitro*.[49] The amount of protection correlated with the dose of rooibos, and an extract concentration of 10 percent reduced the cell transformations to a level similar to the spontaneous level of the controls. Interestingly, green tea in equivalent concentrations did not show any detectable protective effect. In another study, fermented rooibos tea reduced cell damage in live mice that were exposed to irradiation two hours following a single dose of rooibos administered by gastric intubation.[34]

Fermented Rooibos against Brain Lipid Peroxidation: Rats given fermented rooibos tea daily *ad libitum* (free access) from the age of 3 months to 24 months had greatly reduced age-related lipid peroxide accumulation in four areas of their brains compared to rats that drank plain water.[50] Increases in lipid peroxides in the brain may damage neuronal cells and contribute to age-related diseases.[50] The lipid peroxide levels were evaluated by measuring the amounts of thiobarbituric acid reactive substances (TBARS) in eight regions of the brain. The 24-month-old rats that had been drinking plain water had significantly higher TBARS in the frontal cortex, occipital cortex, hippocampus, and cerebellum compared to 5-week-old rats, but the 24-month-old rats that had been drinking rooibos tea had no increase in TBARS in those four areas of the brain. The TBARS of the 24-month-old rooibos group were similar to the TBARS of the young 5-week-old group (see Table 4).

The authors give a bar chart that summarizes the TBARS data for each area of the brain.[50] The TBARS values in nmol/g for 24-month-old rats without rooibos tea, 24-month-old rats given rooibos tea, and 5-week-old rats, respectively, were approximately 120, 80, 80 in the frontal cortex; 115, 70, 80 in the occipital cortex; 80, 40, 50 in the hippocampus; and 115, 80, 85 in the cerebellum. The authors say these results suggest that the administration of rooibos

tea protected several regions of the rat brain against lipid peroxidation accompanying aging. Magnetic resonance images taken of the brain were consistent with the TBARS data.

Fermented vs. Unfermented Rooibos: Another study found that both fermented and unfermented rooibos tea exhibits antimutagenic properties *in vitro* as measured by the *Salmonella typhimurium* mutagenicity assay with several different mutagens; the antimutagenic activity was stronger against the metabolically activated mutagens 2-acetylaminofluorene (2-AAF) and aflatoxin B_1 (AFB_1) than it was against three direct-acting mutagens.[51] Further research showed that the fermentation process causes a decrease in the antimutagenic and antioxidant activity of rooibos tea as measured by the *Salmonella typhimurium* mutagenicity assay (with 2-AAF), the hydrogen donating ability (assessed with DPPH), and the superoxide anion radical scavenging assay.[52] The researchers suggest that fermented rooibos may show less antioxidant and antimutagenic activity because it has less polyphenols than unfermented rooibos. One analysis showed that polyphenols represent about 41 percent of the total solid matter in unfermented rooibos tea extract, but only about 30 percent of the total solid matter in fermented rooibos tea extract.[51]

One of the authors of both these studies is senior research scientist Jeanine Marnewick of the Program on Mycotoxins and Experimental Carcinogenesis (PROMEC) at the Medical Research Council of South Africa. She says, "Rooibos showed protective effects against DNA damage when tested in an *in vitro* assay as well as in an *in vivo* animal system." [53] The *in vitro* studies found unfermented rooibos was generally more protective against DNA damage than fermented rooibos. But Marnewick says her group's research shows that fermented rooibos has a stronger effect against some mutagens. She says, "Both the fermented and unfermented rooibos showed a significant protection, and we're busy elucidating the mechanisms."[53] She is currently evaluating the protective effect of rooibos on liver, esophageal, colon, and skin cancer induced in live animal models. The studies are in the early phases and she cautions, "Very little is known about the effect of rooibos on cancer development." [53]

Joubert also adds a cautionary note, saying that many questions about rooibos still need to be answered.[22] She says that researchers need to determine which of the antioxidant substances in rooibos tea are absorbed by the body and how much tea is needed to produce a measurable benefit. She also emphasizes that no human studies have been conducted yet.

Whole Foods vs. Isolated Antioxidants: The full benefits of teas are likely to come from a combination of all the antioxidants in them rather than from just one substance. Quite a few studies have found that isolated antioxidants don't have as positive an anticancer effect as the mixture of antioxidants found in natural food sources; whole apple extracts were better than pure quercetin at inhibiting the growth of cancer cells *in vitro*,[13,54] tomato powder was better than pure lycopene at extending the life of rats with prostrate tumors,[13,55] and freeze-dried strawberries exhibited better anticancer properties in animals than did pure ellagic acid.[13,56] Also, white and green tea extracts demonstrated better antimutagenic properties *in vitro* than mixtures of nine polyphenols found in the teas (mixed according to their relative proportions in the teas).[57] Researchers believe these results indicate that other substances in the whole food products besides the identified antioxidants probably contribute to the total anticancer effect of the food, and that the relative amounts of all these substances could be important. Different teas have different mixtures of antioxidants, and they will protect against different mutagens. Sorting out all of these interactions will take time.

Rooibos Folklore: What's Proven?

Although rooibos does contain active antioxidants, many of the other health claims made for rooibos tea are not well documented (based only on anecdotal evidence) or are not supported by science. Researchers are still investigating many of these claims to evaluate all the potential benefits of rooibos.

Table 5: Minerals in a 200 ml Cup of Rooibos Tea

Mineral	Amount (mg)	RDI/AI (mg)	% DV
Iron	0.07	8, 18	0.9, 0.4
Potassium	7.12	3500	0.2
Sodium	6.16	<2400	0.3
Calcium	1.09	1000	0.1
Copper	0.07	0.9	7.8
Zinc	0.04	11, 8	0.4, 0.5
Magnesium	1.57	420, 320	0.4, 0.5
Fluoride	0.22	4, 3	5.5, 7.3
Manganese	0.04	2.3, 1.8	1.7, 2.2

Note: RDI is the US reference daily intake. AI is the US adequate intake, which is used when an exact RDI is not well established. Percent DV is the percentage of the daily reference value that is provided by a serving of the food. Values are for adults; when two values are given, the first is for men and the second is for women.

Sources: Values for minerals in mg are from the Rooibos Limited website: <http://www.rooibosltd.co.za>. The RDI/AI data are from the dietary reference intake tables on the US government website: <http://www4.nationalacademics.org>.

Vitamins And Minerals: Despite some promotional claims that rooibos is a source of vitamin C, Joubert says it is not. "We have tested both the traditional rooibos and green rooibos, and vitamin C was not present," she says.[22]

With the exceptions of fluoride and copper, the trace amounts of minerals in rooibos are not enough to make the tea a meaningful dietary source of minerals for the average consumer. As shown in Table 5, the nutritional labeling that is given on some packages of rooibos tea and on some websites of distributors[4,5] indicates that the amounts of iron, potassium, zinc, calcium, and magnesium in a 200 ml serving of rooibos tea are all less than 1 percent of the U.S. reference daily intake (RDI). A 200 ml serving of rooibos provides over 5 percent of the RDI of fluoride for adults and over 7 percent of the RDI for copper (see Table 5). Marc S. Micozzi, M.D., Ph.D., director of the Policy Institute for Integrative Medicine in Bethesda, Maryland, notes that when rooibos is used as a fluid replacement throughout the day, as is done with some athletes in South Africa, it does provide measurable amounts of several minerals and electrolytes.[58]

Colic, Allergies, And Other Ailments: Distributors of rooibos tea often suggest it can help allergies, sleep problems, digestive problems, headache, and other ailments,[4,5] but these claims have not been verified by scientific research. If the indigenous people of the Cedarberg region used rooibos tea medicinally, that tradition was lost and rooibos was just enjoyed as a good-tasting beverage until the recent interest in its health benefits.[10] Many of the health claims for rooibos tea began in 1968 when a South African woman, Annekie Theron, found that rooibos tea eased her infant's colic.[10] As the story goes, she found no documentation on the benefits of rooibos and began her own experiments with local babies who had colic and allergies.[10] She concluded that rooibos helped these babies, and she published a book in 1970 titled *Allergies: An Amazing Discovery*. Since then, she patented a rooibos extract that is now used in cosmetic products, and she started her own line of health and cosmetic products.[10]

Today, South African physicians recommend rooibos for infant colic.[59] South Africans also use it to calm digestive upset in adults, to help induce sound sleep, and topically to sooth eczema, skin allergies, and diaper rash.[59] Not enough research has been done to know if these folk remedies really are effective or to identify the substances in the tea that might be

responsible for any observable benefits. Joubert says the tea does seem to help infant colic, but no formal studies have been done.[22]

Immune Function: An *in vitro* and *in vivo* study showed that rooibos might enhance immune function, but very little research has been done on this topic.[60] One study found that a polysaccharide in rooibos leaves may have antiviral activity against the HIV virus, but the polysaccharide had to be chemically extracted from the leaves and is not found in tea made by steeping the leaves in water.[61] There's no evidence that rooibos tea fights the HIV virus.

Zero Caffeine And Low Tannin: Several other health advantages of rooibos tea that are often mentioned are its zero caffeine content and its low tannin content. Because rooibos is naturally caffeine-free, it does not have to be subjected to a decaffeination process and, therefore, does not lose any of its polyphenol content (as occurs when green and black teas are decaffeinated). The zero caffeine content also means rooibos can be enjoyed by those who want to avoid the stimulating effects of caffeine and can be consumed in quantity by those who want to use it as a fluid replacement.

Rooibos only has about 4.4 percent tannin content,[51] which means that it does not have the astringent taste associated with *C. sinensis* and will not become bitter even after long steeping times. Rooibos tea can be a good alternative to *C. sinensis* for people who prefer the milder taste of a less astringent herbal tea or for those who have digestive problems with tannin-rich beverages. And as Micozzi observes, some people can receive a higher total antioxidant intake from rooibos than from green or black tea because the low tannin content and caffeine-free nature of rooibos allow it to be consumed in larger quantities.[58]

Iron Absorption: Other disadvantages have been attributed to tannins; they can bind to non-heme iron (iron from non-meat sources), reducing iron absorption, and they can decrease the metabolism and utilization of proteins.[62-69] Black and green teas reduce the amount of non-heme iron absorbed by the body when the tea is consumed at the same time as the iron source.[62-66] These effects do not cause problems for most people, but they can cause problems for people who have nutritionally marginal diets or low intake of heme iron sources (meats).[69]

Other polyphenol-rich beverages besides *C. sinensis* teas can also inhibit iron absorption. One study found that the inhibition of iron was 79 to 94 percent for black tea, 84 percent for peppermint tea, 73 percent for hot cocoa, and 47 percent for tea of chamomile (*Matricaria recutita* L., Asteraceae).[62] The teas still inhibited iron absorption to the same degree even if milk was added to them. Some of these beverages contain only low levels of tannins, but other polyphenols in foods and beverages can also reduce iron absorption.[62,64] The ability of polyphenols to chelate prooxidant metal ions might provide some antioxidant protection, but it can also be a disadvantage by decreasing absorption of necessary dietary minerals such as iron.[64]

The low tannin content of rooibos is sometimes used to infer that rooibos tea won't inhibit iron absorption, but that conclusion is not automatic since rooibos is rich in other polyphenols that might decrease iron absorption. In one small study, three groups of 10 young healthy men were given an oral dose of iron, followed by rooibos tea, *C. sinensis* tea, or plain water.[71] Iron absorption was measured to be 7.25 percent for rooibos tea, 1.70 percent for *C. sinensis* tea, and 9.34 percent for plain water. The result for *C. sinensis* was significant ($P < .0001$), but the data for rooibos did not reach statistical significance (that is, the data for rooibos were not good enough to determine whether this result can be generalized to the whole population or whether the result was just chance). More studies are needed to better document the effect of

rooibos on iron absorption, but this study implies that rooibos might not inhibit iron absorption nearly as much as *C. sinensis* tea.

The Bottom Line

Rooibos tea has become popular because of its fruity, sweet taste and its caffeine-free, low tannin, antioxidant-rich status. Although more research is needed, rooibos appears to be safe and free of side effects. The antioxidants present in rooibos may help protect against free radical damage that can lead to cancer, heart attack, and stroke. Unfermented (green) rooibos has a higher amount of polyphenols than traditional fermented rooibos and generally demonstrates higher antioxidant and antimutagenic capabilities *in vitro*. Future research should reveal whether the antioxidant benefits of rooibos observed *in vitro* and in animals translates into health benefits for humans.

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