

## 2. Rooibos tea as a likely health food supplement

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# Rooibos Tea as a Likely Health Food Supplement

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## ABSTRACT

Rooibos tea contains a variety of substances possessing the functional groups that are required for these compounds to act as antioxidants, i.e. scavengers of active oxygen species which adversely affect human health. In addition some of these compounds also exhibit other physiological and therapeutic properties which are beneficial for a healthier life.

## INTRODUCTION

*Aspalathus linearis* is a leguminous shrub indigenous to the Cedarberg mountains around Clanwilliam near Cape Town in South Africa<sup>(1-3)</sup>. Its leaves and fine stems are used for the manufacture of Rooibos tea by cutting into 5 mm length, rolling, fermenting by leaf enzymes and solar drying in process similar to that for black tea or oolong tea. The Rooibos plant is increasingly recognized as one of the relatively few economic plants that has made the transition from a local wild resource to a cultivated crop in the 20th century. In South Africa Rooibos tea is mainly used as a substitute for Oriental black tea by people who enjoy it either hot or cold, or by those who regard it highly as a healthy drink. It is a unique beverage with a characteristic sweet flavour and that is rich in volatile compounds<sup>(4,5)</sup>, minerals and ascorbic acid, is caffeine-free, and is claimed to have a low tannin content (as gallic acid)<sup>(2,4)</sup>. Owing to the absence of deleterious effects of the beverage on human health<sup>(2)</sup>, Rooibos tea is rapidly gaining in popularity as a health beverage.

Clinically, Rooibos tea is often prescribed against nervous tension, allergies and various stomach and indigestive problems. In recent studies, it was established that Rooibos tea possesses antioxidative activity by superoxid dismutase (SOD) mimetic substances<sup>(6,7)</sup> and has effects on

dermatological diseases such as Behcet's disease, Sweet disease and photosensitive dermatitis<sup>(8)</sup>. Previous investigations of the chemical constituents of Rooibos Tea have demonstrated the presence of the flavonol, quercetin and the flavone, luteolin with their known antispasmodic properties<sup>(9,10)</sup>, and five additional flavonoid glycosides, the dihydrochalcone, aspalathin<sup>(11)</sup>, the flavones, orientin and iso-orientin<sup>(11)</sup>, and the flavonol glycosides, isoquercitrin and rutin<sup>(12)</sup>. These phenolic compounds presumably contribute significantly towards the scavenging effects<sup>(13)</sup> of Rooibos tea on active oxygen species. Collectively, this information prompted a comprehensive investigation<sup>(14)</sup>, in alliance with Rooibos Tea Natural Products Ltd., Clanwilliam, of the polyphenols and other metabolites in Rooibos tea that may contribute towards its beneficial effects on human health.

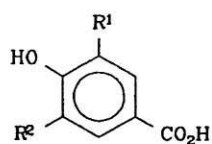
## RESULTS AND DISCUSSION

In order to simulate the composition of the mixture that is reminiscent of a 'cup of Rooibos tea', the aqueous extract of the commercial product, kindly supplied by Rooibos Tea Natural Products Ltd., Clanwilliam, was selected for the current investigation. The plant material was initially extracted with chloroform to remove chlorophyll and subsequently with boiling water to give an aqueous mixture which was successively extracted with hexane (to remove waxy materials), diethyl ether and ethyl acetate. The residual plant material was finally extracted exhaustively with acetone at ambient temperatures. Owing to the complexity of the various extracts, certain fractions had to be derivatized to attain an acceptable level of purity. It should be emphasized that the metabolic pool of the Rooibos provided a mixture of polyphenols and other compounds that is more complex than any of the multitude of natural products mixtures that this research group have been investigating in the past. Our initial approach was divided into three different phases; (i) Comprehensive analysis of the polyphenols/flavonoids and other chemical constituents from the aqueous extract; (ii) A literature survey of the physiological effects of the identified compounds; and (iii) Assessment of the scavenging effects of a selection of these compounds on 'active oxygen species'.

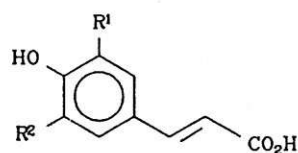
### Chemical Constituents of Rooibos Tea

#### Phenolic Carboxylic Acids

The aforementioned ether extract afforded the phenolic carboxylic acids, 4-hydroxybenzoic acid (1), 3,4-dihydroxybenzoic acid (protocatechuic acid) (2), 4-hydroxy-3-methoxybenzoic acid (vanillic



- 1  $R^1=R^2=H$   
 2  $R^1=OH, R^2=H$   
 3  $R^1=OCH_3, R^2=H$   
 4  $R^1=R^2=OCH_3$



- 5  $R^1=R^2=H$   
 6  $R^1=OH, R^2=H$   
 7  $R^1=OCH_3, R^2=H$   
 8  $R^1=R^2=OCH_3$

acid) (3), and 4-hydroxy-3,5-dimethoxybenzoic acid (syringic acid) (4), and the hydroxycinnamic acids, 4-coumaric acid (5), caffeic acid (6) and ferulic acid (7), while the 4-hydroxy-3,5-dimethoxycinnamic acid (8) was present in the ethyl acetate fraction. The presence of these  $C_6 \cdot C_3$  acids (5), (6), (7) and (8) gives credence to the central position of activated hydroxycinnamic acids,

Table 1. Physiological and therapeutic properties of phenolic carboxylic acids (1)-(8) in Rooibos tea

Activity	Active Compounds							
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8 <sup>a</sup> )
Antibacterial		*				*	*	*
Antifungal		*			*	*	*	*
Anti-yeast							*	*
Antiviral		*				*		
Anthelmintic			*					
Antiarrhythmic							*	
Reduce myocardial O <sub>2</sub> consumption		*						
Antihepatotoxic		*			*	*	*	*
Antimitotic							*	
Antitumor							*	
Antimutagenic	*							
DNA-binding effect						*		
Gene conversion						*		
Anti-oestrogenic							*	
Antioxidant		*		*		*	*	
Antithiamine						*		
Phagocytosis stimulant							*	
Platelet aggregation inhibition						*	*	
Prolactin stimulant							*	
Spasmolytic	*							
Clastogenic						*		
Anti-ulcer						*		

<sup>a</sup>As sinapic acid

*i.e.* as CoA esters, in the biosynthesis of various phenylpropanoid metabolites<sup>(15)</sup>. The anti-microbial properties of hydroxybenzoic acids have been firmly established<sup>(16–18)</sup>, thus, compounds (1)–(4) may function as natural preservatives in Rooibos tea. Table 1 lists the claimed physiological and therapeutic properties<sup>(18–21)</sup> of the above carboxylic acids. Notable from Table 1 is the antioxidative properties of those acids with *o*-dihydroxy functionality, *e.g.* protocatechuic acid (2). Antioxidative activity of these acids decreases in the order protocatechuic acid (2) > caffeic acid (6) > 4-hydroxybenzoic acid (1) > ferulic acid (7) > vanillic acid (3) > syringic acid (4) > *p*-coumaric acid (5)<sup>(21)</sup> (see also below).

#### Flavones, Flavonols and C-O-glycosides

In addition to the aforementioned carboxylic acids the ether and ethyl acetate extracts also contained the flavones, 3',4',5,7-tetrahydroxyflavone (luteolin) (9), 4',5,7-trihydroxy-3'-methoxyfla-

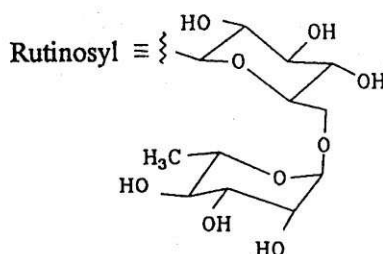
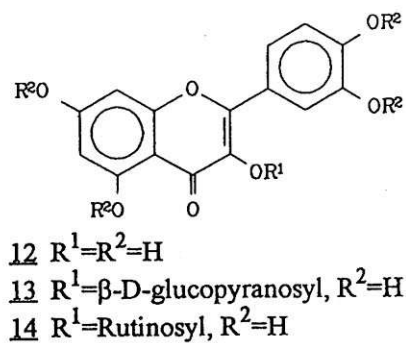
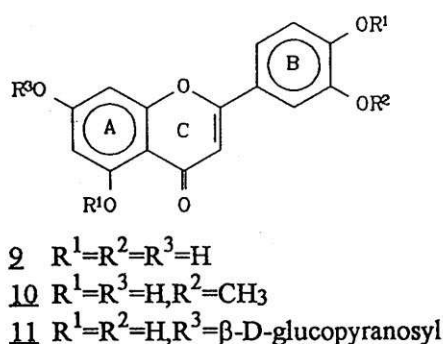


Table 2. Physiological and therapeutic properties of compounds (9) and (12)–(14)

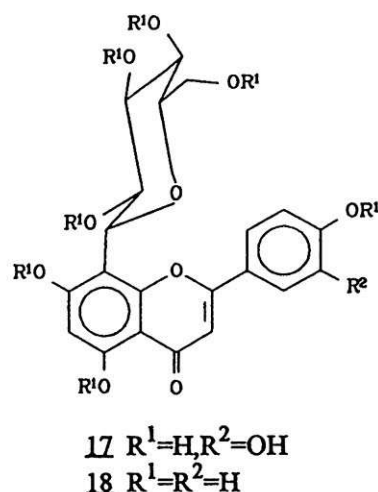
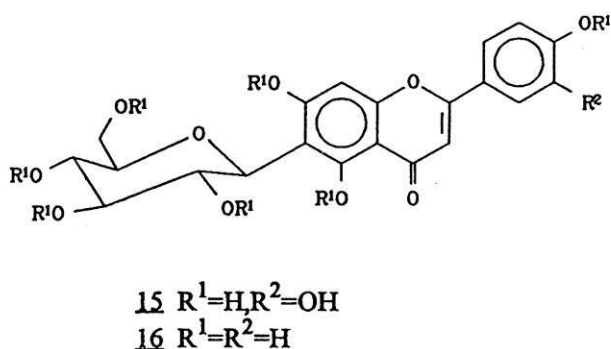
Activity	Active compounds			
	(9)	(12)	(13)	(14)
Antioxidant	*	*	*	*
Antispasmodic	*	*		
Antineoplastic		*		*
Antiviral		*		
Inhibition of calcium transport systems		*		
Vitamin P activity				*

vone (chrysoeriol) (**10**), luteolin-7-O- $\beta$ -D-glucopyranoside (**11**), the flavonol, 3',4',5,7-tetrahydroxyflavonol (quercetin) (**12**), its 3-O- $\beta$ -D-glucopyranoside derivative (isoquercitrin) (**13**) and the quercetin-3-O-rutinoside (rutin) (**14**). Their physiological and therapeutic properties<sup>(22-29)</sup> are collated in Table 2.

Luteolin (**9**) and quercetin (**12**) with their known anti-spasmodic properties<sup>(9,10)</sup> were among the first physiologically active compounds to be isolated from Rooibos Tea. The glycosides isoquercitrin (**13**) and rutin (**14**) are, however, equally important as far as anti-oxidant properties are concerned. Rutin is of special importance due to its pharmacodynamic properties. Owing to its established vitamin P (P $\equiv$ permeability) activity, it enhances the stability and permeability of capillary arteries. Rutin occurs abundantly in nature and is thus included in a variety of medical formulations<sup>(30)</sup>. Furthermore, luteolin (**9**), quercetin (**12**), isoquercitrin (**13**) and rutin (**14**) inhibit aldose reductase which is considered to be a target enzyme for pharmacological control of diabetic complications<sup>(31)</sup>. The oxidation of low-density lipoproteins (LDL) is also prevented by quercetin which may indicate anti-atherosclerotic activity.

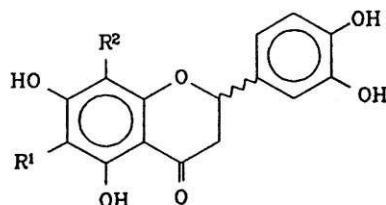
#### C-C-linked Flavone Glycosides

Although the 3',4',5,7-tetrahydroxy-6-C- $\beta$ -D-glucopyranosylflavone, iso-orientin (**15**)<sup>(11)</sup>, its C-8 isomer orientin (**17**)<sup>(11)</sup>, and the 4'-deoxy analogues, iso-vitexin (**16**) and vitexin (**18**) as well as their glycosidic derivatives exhibit a wide taxonomic distribution<sup>(33)</sup> the co-existence of iso-vitexin and vitexin with iso-orientin and orientin in Rooibos tea, has only recently been demonstrated<sup>(14)</sup>. Although the 2''-O-xylosyl derivative of vitexin showed strong hypotensive activity<sup>(34,35)</sup>, little is known in connection with the physiological and therapeutic properties of the four C-C-linked flavone glycosides (**15**)-(18).



### C-C-linked Flavanone Glycosides

The 3',4',5,7-tetrahydroxy-6-C- $\beta$ -D-glucopyranosylflavanone, dihydro-iso-orientin (**19**), and its C-8 isomer, dihydro-orientin (**20**) represents entries among this of natural products that are



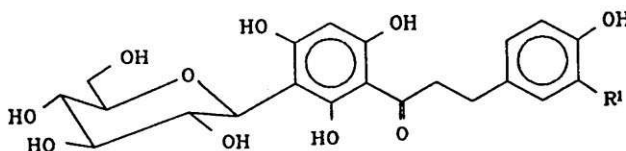
**19**  $R^1 = \text{C-}\beta\text{-D-glucopyranosyl}, R^2 = \text{H}$

**20**  $R^1 = \text{H}, R^2 = \text{C-}\beta\text{-D-glucopyranosyl}$

to *A. linearis*. We have not yet succeeded in establishing the absolute stereochemistry at C-2 of compounds **19** and **20**. Both these compounds possess structural features that are essential for chemicals acting as non-nutritional sweeteners<sup>(36-38)</sup>. The pleasant sweet natural taste of Rooibos tea, in contrast to the characteristic bitter taste of Oriental black tea, may presumably in part be attributed to the presence of these and related compounds (*vide infra*).

### C-C-linked Dihydrochalcone Glycosides

The flavonid composition of *A. linearis* is unique in the sense that it is hitherto the only natural source containing the C-C-linked 2',3,4,4',6'-pentahydroxy-3'-C- $\beta$ -D-glucopyranosyldihydro-



**21**  $R^1 = \text{OH}$

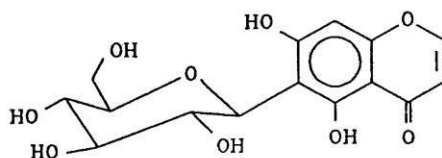
**22**  $R^1 = \text{H}$

rochalcone, aspalathin (**21**)<sup>(11)</sup>, the main component in the unprocessed material. Rooibos tea also contains nothofagin (**22**), another rare polyhydroxydihydrochalcone<sup>(39)</sup> that has previously only been isolated<sup>(40)</sup> from *Nothofagus fusca*. Aspalathin (**21**) and nothofagin (**22**) constitute *ca.* 0.55% and 0.9% of the soluble solids of the processed tea<sup>(39)</sup>. It was furthermore demonstrated<sup>(39)</sup> that the aspalathin content is reduced significantly during processing. We suspect that aspalathin (**21**) is enzymatically oxidized to the flavanones, dihydro-iso-orientin (**19**) and dihydro-orientin (**20**) during the manufacturing process and are currently attempting to formulate a biomimetic model for the fermentation process that is based upon this oxidative transformation.

The utilization of certain dihydrochalcone derivatives and related compounds as sweetening agents has been reviewed recently<sup>(41)</sup>. Studies in this regard have focussed on the structure of the sweet-sensing receptor site and hence the structural features that are essential for a 'fit' of the active compound to the shape and/or size of the binding site. 2',3,4',6'-Tetrahydroxy-4-methoxydihydrochalcone-4'-O-neohesperidoside is indeed commercially used as sweetening agent and its crystal structure has been studied<sup>(42)</sup> in an attempt to gain insight into the chemistry of non-nutritional sweetening agents. Aspalathin (**21**) and nothofagin (**22**) may thus also contribute to the natural sweet taste of Rooibos tea.

#### A C-C-linked Chromone Glycoside

Another novel compound, 5,7-dihydroxy-6-C- $\beta$ -D-glucopyrnosylchromone (**23**) has recently been identified in Rooibos tea. Chromones are considered as products of *post mortem* processes



**23**

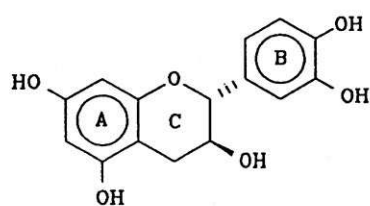
*via* phenol oxidation of 4'-hydroxyflavanones. Thus, chromone (**23**) may originate by the oxidative conversion of dihydro-iso-orientin (**19**) during the fermentation stage of the manufacturing process.

#### Condensed Tannin-type Compounds

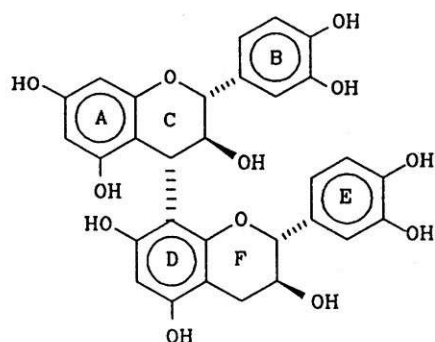
Our current investigation of the polyphenols of Rooibos tea has hitherto confirmed the presence of three condensed-tannin type metabolites, *i.e.* catechin (**24**), the chain terminating flavan-3-ol unit in the biosynthetic process leading to this class of natural products, procyanidin B3 (**25**), and the profisetidin triflavanoid, bis-fisetidinol-(4 $\beta$ ,6:4 $\beta$ B,8)-catechin (**26**). However, the extreme low concentrations of these compounds indeed give credence to the claim that Rooibos tea has a low tannin content<sup>(2,4)</sup>.

Despite convincing evidence<sup>(45)</sup> that condensed tannins have an adverse effect of human and animal feeding by reducing the digestibility of proteins via complexation, the beneficial effects on human health of low concentrations of tannins in the human diet is also well documented<sup>(46,47)</sup>. Catechin (**24**) is indeed utilized in a variety of formulations for use by humans<sup>(20,48,49)</sup>.

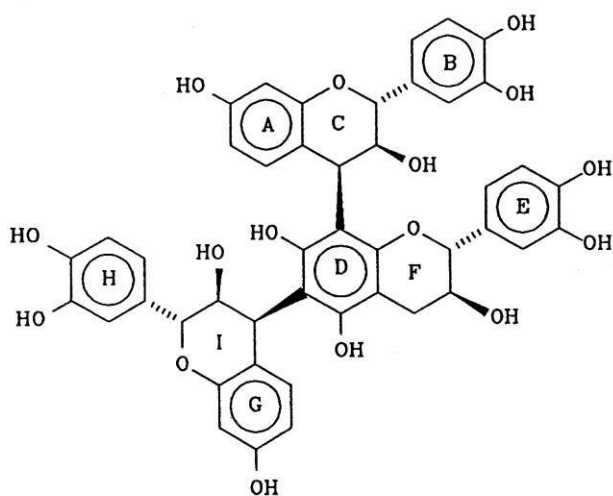




**24**



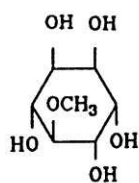
**25**



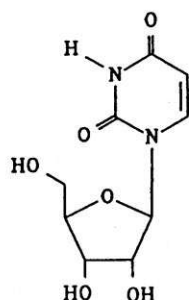
**26**

### Non-phenolic Metabolites

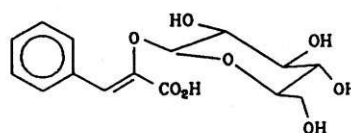
The non-phenolic metabolites from Rooibos tea comprise the inositol, (+)-pinitol (**27**), the nucleoside, uridine (**28**), and the phenylpyruvic acid-O- $\beta$ -D-glucopyranosyl derivative (**29**). (+)-Pinitol is one of the most abundant cyclitols in nature<sup>(50,51)</sup>. The inositols and their phosphates fulfill an important role in cellular communication and are claimed to possess antglycosidic-



**27**



**28**



**29**

and antiviral properties<sup>(25-55)</sup>. More significantly, however, is the considerable hypoglycemic and antidiabetic activities<sup>(56)</sup> and the use of (+)-pinitol as an expectorant.

Uridine (28) is one of the four nucleosides that is present in the nucleic acid, RNA which is responsible for protein transcription. Several nucleosides have been applied in cancer chemotherapy<sup>(57)</sup>, especially the C-fluoro derivatives. Uridine (28) itself is applied successfully in treatment of the symptoms of hereditary orotic aciduria<sup>(58)</sup>, a disease that is caused by a deficient pyrimidine metabolism.

The unique metabolic pool of *A. linearis* is additionally complemented by compound (29), the first naturally occurring glycoside of phenylpyruvic acid. The exceptionally unstable phenylpyruvic acid is presumably stabilized by formation of the enolic O- $\beta$ -D-glucopyranoside (29) thus ensuring availability of the aglycone in specific metabolic processes. Phenylpyruvic acid indeed plays a key role in the biosynthesis of the C<sub>3</sub>·C<sub>6</sub> portion of the C<sub>6</sub>·C<sub>3</sub>·C<sub>6</sub> backbone of flavonoids<sup>(59)</sup> and is presumably the most likely precursor of  $\alpha$ -hydroxychalcones<sup>(60)</sup> and hence to flavonoids possessing 3-oxygenation of ring C.  $\alpha$ -Hydroxy- and  $\alpha$ -keto-acids including phenylpyruvic acid are used for the prevention of and in therapeutic preparations for dermatological diseases<sup>(61)</sup>.

## THE FLAVONOIDS OF ROOIBOS TEA AS SCAVENGERS OF 'ACTIVE OXYGEN SPECIES'

### 'Active Oxygen Species' and Their Adverse Effects on Human Health

Although molecular oxygen (O<sub>2</sub>) is essential for life, its normal metabolism results in the formation of free radicals, *e.g.* superoxides and peroxides, that are detrimental to human health<sup>(62)</sup>. The toxicity of superoxide and hydrogen peroxide are related to their *in vivo* transformation into the highly active hydroxyl radical (*vide infra*) in the presence of suitable transition metals, *e.g.* iron<sup>(63)</sup>. This radical indiscriminately attacks lipids, proteins and DNA<sup>(64)</sup>. Free radical damage is manifested in lipid peroxidation, protein denaturation and DNA mutation *via* attack of the radicals on different substances in living tissues and cells<sup>(65)</sup>. Singlet oxygen, another reactive oxygen species that is formed both in the lens and retina of the mammalian eye, can attack lipids to cause lipid peroxidation. Usually the body can cope with these harmful effects as antioxidant defense enzymes and antioxidant nutrients like vitamins A, C and E protect the body against oxidative substances<sup>(66)</sup>. When the radical defense mechanisms fail or are weakened *e.g.* as a result of ageing<sup>(67)</sup>, and/or inadequate nutrition<sup>(68)</sup>, oxidative stress occurs with serious consequences to human health. An imbalance in the oxidative levels is believed to be a contributing factor in a broad spectrum of diseases including atherosclerosis, inflammatory diseases such

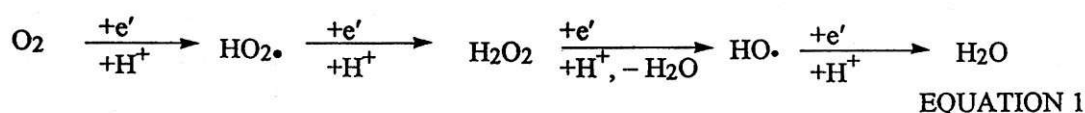
as arthritis, heart disease, Alzheimer's disease, various cancers and even AIDS.

Beside constant attack of the human body by free radicals, the food of Man is also susceptible to oxidative changes resulting in the formation of off-flavours, odours and potentially toxic by-products, pigment discoloration, changes in texture and reduction in nutritional values which ultimately limit the shelf life of foods<sup>(69)</sup>.

The electron configuration of the different states of oxygen may be presented as follows: Oxygen is paramagnetic and has biradical character in the triplet or ground state. Triplet oxygen is not very reactive but is easily transformed into the labile singlet oxygen which exists in the two forms  $^1\Delta_g$  and  $^1\Sigma_g^+$ .

Name	Triplet oxygen (Ground state)	Singlet oxygen	Singlet oxygen	Superoxide
Symbol	$^3O_2$ $^3\Sigma_g^-$	$^1O_2$ $^1\Delta_g$	$^1O_2$ $^1\Sigma_g^+$	$O_2^{\cdot -}$
Electron Configuration	$\pi_{2p}^* \uparrow \uparrow$ $\pi_{2p} \uparrow \downarrow \uparrow \downarrow$ $\sigma_{2p} \uparrow \downarrow$ $\sigma_{2p}^* \uparrow \downarrow$ $\sigma_{2s} \uparrow \downarrow$ $\sigma_{1s}^* \uparrow \downarrow$ $\sigma_{1s} \uparrow \downarrow$	$\uparrow \downarrow$ $\uparrow \downarrow \uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$	$\uparrow \downarrow$ $\uparrow \downarrow \uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$	$\uparrow \downarrow \uparrow$ $\uparrow \downarrow \uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$ $\uparrow \downarrow$

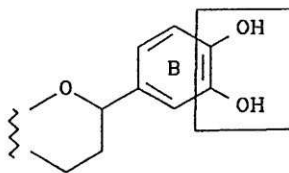
The reduction of oxygen to water requires the transfer of four electrons and involves several reactive intermediates including the hydroperoxy radical ( $HO_2\cdot$ ) or its ionized form, the superoxide



xide species ( $O_2^{\cdot -}$ ). These give rise to the relatively stable hydrogen peroxide ( $H_2O_2$ ) which is further reduced to the highly reactive hydroxyl radical ( $\cdot OH$ ). This radical reacts oxidatively with biological material and causes the so-called oxygen-poisoning<sup>(64)</sup>. Although both the superoxide radical ( $O_2^{\cdot -}$ ) and hydrogen peroxide ( $H_2O_2$ ) are both relatively stable, their reactivity manifests itself in the ability to form the highly reactive hydroxyl radical ( $\cdot OH$ ).

### Phytochemicals as Antioxidants

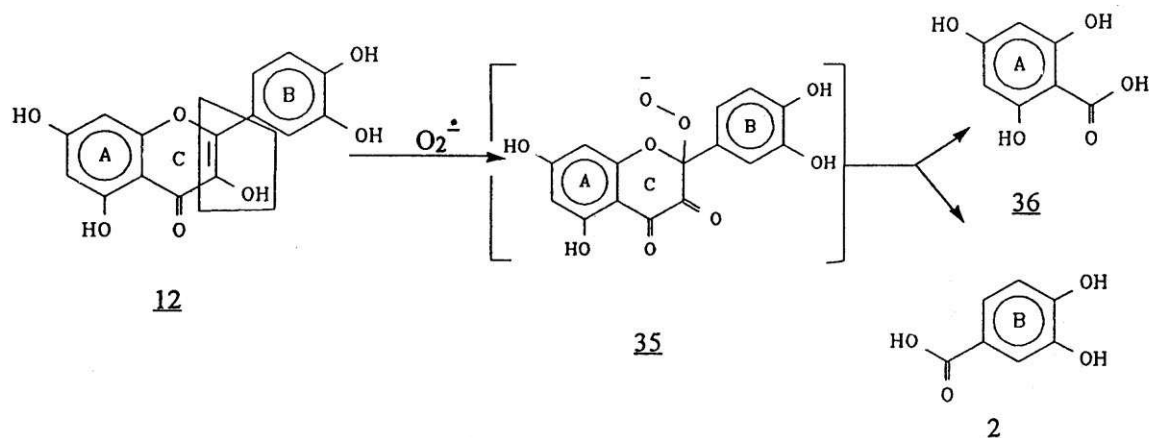
Nature provides an abundance of antioxidants to protect the human body and food against



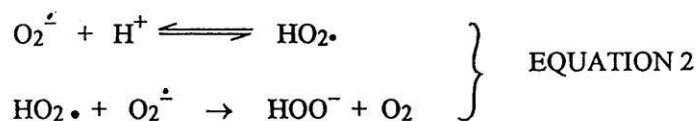
**34**

techuic acid (**2**) and caffeic acid (**6**). Based upon fundamental chemical principles, the ene-diol functionality in the electron-rich aromatic B-ring system (**34**) should be considerably more susceptible to oxidation than the relatively electron deficient system in the  $\alpha$ ,  $\beta$ -unsaturated  $\gamma$ -lactone arrangement of vitamin C (**31**). The aforementioned flavanoids and phenolic acids should, in principle, thus be excellent suppliers of the electrons that are required in EQUATION 1 for the reduction of the active oxygen species to water.

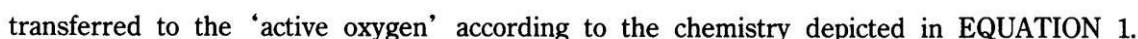
Very recently, it has been demonstrated<sup>(79)</sup> that flavonols, *e.g.* quercetin (**12**), are oxidized by superoxide ( $O_2^{\cdot-}$ ) in heterogenous aprotic media to the carboxylic acids (**36**) and (**2**) *via* the peroxy intermediate (**35**). The enol functionality of the C-ring, boxed in structure (**12**), is a



prerequisite for this transformation. Of even greater significance is the findings of the same authors that other flavonoids like flavones and flavanones induce only the disproportionation of the superoxide anion (equation 2), without undergoing further oxidation. These flavonoids, represented by luteolin (**9**), chrysoiriol (**10**), luteolin-7-O- $\beta$ -D-glucopyranoside(**11**), the orientins (**15**) and (**17**) and vitexins (**16**) and (**17**) and the dihydro-orientins (**19**) and (**20**), and presumably



Vitamin E (**30**) is one of the best quenchers of singlet oxygen ( $^1\text{O}_2$ )<sup>(78)</sup> and reacts also with superoxide ( $\text{O}_2^{\cdot-}$ ) while vitamin C (ascorbic acid) (**31**) is not only capable of reducing two equivalents of superoxide ( $\text{O}_2^{\cdot-}$ ) but can quench both peroxy ( $\text{HO}_2\cdot$ ) and hydroxyl ( $\cdot\text{OH}$ ) radicals as well as singlet oxygen ( $^1\text{O}_2$ )<sup>(64)</sup>. The ene-diol functionality in vitamin C [boxed in structure (**31**)] plays an important role in the scavenging effect of the ‘active oxygens’ as may be illustrated as follows. The two electrons that are lost in the successive steps [(**31**)-(**32**)-(**33**)] are then



It has been claimed<sup>(79)</sup> that all flavonoids with 3',4'-dihydroxy functionality of their B-rings [see the structure of luteolin (9)] possess antioxidant activity and hence the potential to scavenge 'active oxygen species'. This ene-diol functionality (34) is present in luteolin (9), quercetin (12), isoquercitrin (13), rutin (14), iso-orientin (15), orientin (17), and the related flavanones, dihydro-iso-orientin (19) and dihydro-orientin (20), aspalathin (21), catechin (24), procyanidin B<sub>3</sub> (25) and the profisetinidin triflavanoid (26) which constitute the twelve main flavonoid-type substances in Rooibos tea (*vide supra*) and is very similar to the same functionality in vitamin C (31). The same dihydroxy arrangement is also present in two of the carboxylic acids, protoca-

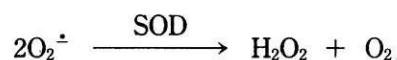
free radical damage<sup>(70,71)</sup>. The use of natural antioxidants in foods for stabilization against oxidative changes is gaining wide acceptance as consumer resistance to synthetic antioxidants is gradually increasing. Antioxidants have a similar 'preservative' effect on biological systems and more specifically on human life. In the past research was focussed mainly on  $\beta$ -carotene, vitamin C and vitamin E but scientists are progressively beginning to realize the potential of other dietary substances, *e.g.* flavonoids, which could, in principle, be incorporated into experimental food.

A specific group of phytochemicals that is of interest to the tea drinker is the theaflavins and thearubigins which are polyphenolic flavan-3-ol oligomers and which play a key role in the quality of black tea<sup>(72)</sup>. The flavonoids act mainly as potent primary antioxidants<sup>(73)</sup> with the ability to scavenge superoxide<sup>(23)</sup>, hydroxyl<sup>(74)</sup> and peroxyl-radical<sup>(75)</sup>. Flavonoids also display secondary antioxidant activity due to their metal-chelating ability<sup>(22)</sup> and quenching of singlet oxygen<sup>(76)</sup>. This group of natural products occurs widely in nature and is therefore an integral part of the human diet. The estimated daily intake of flavonoids in human diet through consumption of plant foods is 1 g. Black tea contributes *ca* 48% of the dietary flavonoids with quercetin a major contributor<sup>(77)</sup>.

While these data may give an indication of the quantity of flavonoids that humans consume, recommendations regarding daily allowances for antioxidants do not consist. Antioxidant requirements of humans are determined by factors such as fat intake, life-style, age, smoking, alcohol intake, infections, occupation, etc. that influence oxidative stress levels.

### **The Quenching of 'Active Oxygen Species'**

The human body is protected against 'active oxygen species' especially by superoxide dismutase (SOD), an 'enzyme' that is capable of quenching an excess of superoxides.



However, when superoxides are produced in an abnormally high concentration or when Man passes the age of about 40 years, the SOD present in a normal human body is not capable of adequately removing the additional supply of superoxides and thus needs to be replenished. This presumably explains why victims of the three major adult diseases apoplexy, myocardial infarction and cancer, are found more among persons beyond the age of 40 whose SOD production and its vital energy have deteriorated. The use of food and drink containing substances with antioxidative properties (bio-antioxidative foods) is thus rapidly gaining in popularity. It

also by aspalathin (21), in Rooibos tea, are therefore antioxidants with undisputed potential and thus of particular interest. We are currently assessing the antioxidant activity of aspalathin (21), the main flavonoid-type constituent of Rooibos tea. The presence of the aforementioned flavonoids and phenolic carboxylic acids in Rooibos tea possessing the structural features that are essential for showing antioxidant activity, when taken in conjunction with its considerable vitamin C content<sup>(2,4)</sup>, unequivocally underline the enormous potential of this renewable natural source as a bio-antioxidative foodstuff. Our present and future research in this regard are thus focussed on the quantification of an 'antioxidative factor' for a cup of Rooibos tea. This would have the obvious advantage that the daily intake of this healthy beverage could be determined by sound scientific reasoning to supplement the human body for SOD-like substances. The growing body of evidence pointing towards the therapeutic value of Rooibos tea gives a considerable degree of credibility to the 'anti-ageing' claims, but expectations of a healthier life rather than an increasing lifespan would perhaps be a more realistic outlook.

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