

Rooibos Tea as a likely Health Food Supplement

Prof Daneel Ferreira a (D.Sc./H.O.D./D.I.C.), Mev. Charlene Marais (M.Sc.), Dr Jacobus A. Steenkamp a (Ph.D.) and Elizabeth Joubert b (Ph.D.).

a Department of Chemistry, University of the Orange Free State, P O Box 339, Bloemfontein, 9300 South Africa; b Infruitec, Private Bag X5013, Stellenbosch, 7599 South Africa.

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.

1. INTRODUCTION

Aspalathus linearis is a leguminous shrub indigenous to the Cedarberg mountains around Clanwilliam near Cape Town in South Africa 1-3 . 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 a 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 4,5, minerals and ascorbic acid, is caffeine-free, and is claimed to have a low tannin content (as gallic acid) 2,4 . Owing to the absence of deleterious effects of the beverage on human health 2, 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 superoxide dismutase (SOD) mimetic substances 6,7 and has effects on dermatological diseases such as Behcet's disease, Sweet disease and photosensitive dermatitis 8 . 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 9,10, and five additional flavonoid glycosides, the dihydrochalcone, aspalathin 11, the flavones, orientin and isoorientin 11, and the flavonol glycosides, isoquercitrin and rutin 12 . These phenolic compounds presumably contribute significantly towards the scavenging effects 13 of Rooibos Tea on active oxygen species. Collectively, this information prompted a comprehensive investigation 14, 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.

2. 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 to remove chlorophyll and the waxy materials. The residual plant material was finally extracted exhaustively with specific solvents 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. The 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'.

2.1 Chemical Constituents of Rooibos Tea

2.1.1 Phenolic Carboxylic Acids

The aforementioned extract afforded 8 different phenolic carboxylic acids. Table 1 lists the claimed physiological and therapeutic properties 18-21 of the above carboxylic acids.

Table 1. Physiological and Therapeutic Properties of Phenolic Carboxylic Acids 1 - 8 in Rooibos Tea

2.1.2 Flavones, Flavonols and C-O-glycosides

In addition to the aforementioned carboxylic acids the four already documented flavanols and two flavones were identified of which the claimed physiological and therapeutic properties are listed in Table 2 .

Table 2. Physiological and Therapeutic Properties of Compounds 9 and 12 - 14.

<Picture: Info Table>

Luteolin (9) and quercetin (12) with their known anti-spasmodic properties 9,10 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 ant-oxidant properties are concerned. Rutin is of special importance due to its pharmacodynamic properties. Owing to its established vitamin P (P = 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 30 . Furthermore, luteolin (9), quercetin (12), isoqueritrin (13), and rutin (14) inhibit aldose reductase which is considered to be a target enzyme for pharmacological control of diabetic complications 31 . The oxidation of low density lipoproteins (LDL) is also prevented by quercetin which may indicate anti-atherosclerotic activity.

2.1.3 C-C-linked Flavone Glycosides

The four identified flavone glycosides in the extracts, as well as their glycosidic derivatives exhibit a wide taxonomic distribution 33, the co-existence of iso-vitexin and vitexin with iso-orientin and orientin in Rooibos Tea, has only recently been demonstrated 14 .

2.1.4 C-C-linked Flavanone Glycosides

The two identified flavanones represents entries among this class of natural products that are unique to *A. linearis*. There have not yet been succeeded in establishing the absolute stereochemistry of the two compounds. Both these compounds possess structural features that are essential for chemicals acting as non-nutritional sweeteners 36-38 . 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.

2.1.5 C-C-linked Dihydrochalcone Glycosides

The flavonoid composition of *A. linearis* is unique in the sense that it is hitherto the only natural source also containing two of these compounds, especially the unique aspalathin 11, the main component in the unprocessed material. Rooibos Tea also contains nothofagin, another rare polyhydroxydihydrochalcone 39 that has previously only been isolated 40 from *Nothofagus fusca*. Aspalathin and nothofagin constitute ca. 0.55% and 0.19% of the soluble solids of the processed tea 39 . It was furthermore demonstrated 39 that the aspalathin content oxidized to the flavanones, during the manufacturing process and are currently attempting to formulate a biomimetic model for the fermentation process that is based upon this oxidative transformation.

2.1.6 Other compounds identified

The unique metabolic pool is further complemented by the identification of two C-C-linked chromone glycosides, three condensed tannin-type compounds, three non-phenolic metabolites and the first naturally occurring glycoside of phenylpyruvic acid.

3. THE FLAVONOIDS OF ROOIBOS TEA AS SCAVENGERS OF 'ACTIVE OXYGEN SPECIES'

3.1 'Active Oxygen Species' and their Adverse Effects on Human Health

Although molecular oxygen (O₂) 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 62 . 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 63 . This radical indiscriminately attacks lipids, proteins and DNA 64 . 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 65 . 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 66 . When the radical defense mechanisms fail or are weakened e.g. as a result of ageing 67 and/or inadequate nutrition 68, 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 69 .

3.2 Phytochemicals as Antioxidants

Nature provides an abundance of antioxidants to protect the human body and food against free radical damage 70, 71 . 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 focused mainly on B-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 72 . The flavonoids act mainly as potent primary antioxidants 73 with the ability to scavenge super oxide 23, hydroxyl- 74 and peroxy-radical 75 . Flavonoids also display secondary antioxidant activity due to their metal-chelating ability 22 and quenching of singlet oxygen 76 . 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 1g. Black tea contributes ca48% of the dietary flavonoids with quercetin a major contributor 77 . While these data may give an indication of the quantity of flavonoids that humans consume, recommendations regarding daily allowances for antioxidants do not exist. 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.

3.3 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 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 may be administered orally in contrast with SOD and liposome-SOD (LSOD) which have to be administered intravenously.

3.4 The Flavonoids of Rooibos Tea and Potential SOD Agents

The ene-diol functionality is present in the twelve main flavonoid-type substances in Rooibos teas (*vide supra*) and is very similar to the same functionality in vitamin C. The same dihydroxy arrangement is also present in two of the carboxylic acids. Based upon fundamental chemical principles, the ene-diol functionality in the electron-rich aromatic B-ring system should be considerably more susceptible to oxidation than the relatively electron deficient system in vitamin C. The aforementioned flavonoids and phenolic carboxylic 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 demonstrated⁷⁹ that flavonols, e.g. quercetin, are oxidized by superoxide (O₂⁻) in heterogenous aprotic media to the carboxylic acids and via the proxy intermediate. 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.

The identified flavonoids, and presumably also aspalathin, in Rooibos Tea, are therefore antioxidants with undisputed potential and thus of particular interest. We are currently assessing the antioxidant activity of aspalathin, 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^{2,4}, unequivocally underline the enormous potential of this renewable natural source as bio-antioxidative foodstuff. Our present and future research in this regard are thus focused 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 need of the human body for SOD-like substances. The growing body of evidence pointing towards the therapeutic value of Rooibos Tea give 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.

ACKNOWLEDGEMENTS

Financial support by The Foundation for Research Development, Pretoria, Die Sentrale Navorsingsfonds of this University and by Rooibos Tea Natural Products Ltd, Clanwilliam is gratefully acknowledged.

REFERENCES

- R. Dahlgren, *Bot. Notiser*, 1968, 121, 165 .
- J.F. Morton, *Econ. Bot.*, 1983, 37, 164 .
- M.E. Komaitis, in *Off-flavors in Foods and Beverage*, ed. G. Charalambous, Elsevier Science Publishers, Amsterdam, 1992, 417 .
- T. Habu, R.A. Flath, T.R. Mon and J.F. Morton, *J. Agric. Food Chem.*, 1985, 33, 249 .
- M. Kawakami, A. Kobayashi and K. Kator, *J. Agric. Food Chem.*, 1993, 41, 663 .
- T. Yoshikawa, Y. Naito, H. Oyamada, S. Ueda, S. Tanigawa, T. Takemura, S. Sugino and M. Kondo, in *Antioxidants in Therapy and Preventive Medicine*, ed. I. Emeril, Plenum Press, New York, 1990, 171 .
- Ito, K. Shinohara and K. Kator, in *Proceedings of the International Symposium on Tea Science*, The

Organizing Committee of ISTS, Shizuoka, 1991, 381 .

- Y Shindo and K. Kator, *ibid*, 385 .
- S. Shibata, M. Harada and W. Budidarmu, *Yakugaku Zasshi*, 1960, 80, 620 .
- F.O. Snyckers and G. Salemi, *J. South African Chem. Inst.*, 1974, 27, 5 .
- B.H. Koeppen and D.G. Roux, *Biochem. J.* 1965, 97, 444; 1966, 99, 604 .
- B.H. Koeppen, M.Sc. Thesis, University of Stellenbosch, South Africa, 1959 .
- J. Robak and R.J. Gryglawski, *Biochem. Pharmacol.*, 1988, 37, 837 .
- Rabe, J.A. Steenkamp, E. Joubert, J.F.W. Burger and D. Ferreira, *Phytochemistry*, 1994, 35, 1559 .
- W. Heller and G. Forkmann in *The Flavonoids - Advances in Research since 1980*, ed. J.B. Harbone, Chapman and Hall, London, 1988, 399 .
- T. Eklund, *Int. J. Food Microbiol.*, 1985, 2, 159 .
- L.V. Bui and C. Cooper, *J. Assoc. Off. Analyt. Chem.*, 1987, 70, 892 .
- M. Kohno, M. Yamada, K. Mitsuta, Y. Mizuta and T. Yoshikawa, *Bull. Chem. Soc., Jpn.*, 1991, 64, 1447 .
- R.A. Larson, *Phytochemistry*, 1988, 27, 969 .
- C.W.W. Beecher, N.R. Farnsworth and C. Gyllenhaal in *Natural Products of Woody Plants II*, ed. J.W. Rowe, Springer-Verlag, Berlin, 1989, 1070 .
- S.N. Onyeneho and N.S. Hettiarachy, *J. Agric. Food Chem.*, 1992, 40, 1496 .
- B.J.F. Hudson and J.I. Lewis, *Food Chem.*, 1983, 10, 47 .
- H. Ogawaram, T. Akiyama, S. Watanabe, N. Ito, M. Kobori and Y. Seoda, *J. Antibiot.*, 1989, XLII, 340 .
- 24.B.D.M. Cunningham, M.D. Treadgill and J.A. Hickman, *British J. of Cancer*, 1987, 56, 207 .
- Y. Inouye, K. Yamaguchi, Y. Take and S. Nakamura, *J. Antibiot.*, 1989, XLII, 1523 .
- M.G.L. Hertzog, P.C.H. Hollman and B. van de Putte, *J. Agric. Food Chem.*, 1993, 41, 1242
- W. Worthy, *Chem & Eng. News*, 16 Sept. 1991, 27 .
- Barzilai and H. Rahamimoff, *Israel J. Med. Sc.*, 1982, 18, 2 .
- H. Okamura, A. Mimura, Y. Yakou, M. Niwano and Y. Takahara, *Phytochemistry*, 1993, 33, 557 .
- K. Herrman, *J. Food Technol.*, 1976, 11, 433 .
- S.D. Varma and *Plant Flavonoids in Biology and Medicine*, eds. V. Cody, E. Middleton and J.B. Harborne, Alan R. Liss, Inc., New York, 1986, 343.
- C.V. de Whalley, S.M. Rankin, J.R.S. Hoult, W. Jessup and D.S. Leake, *Biochem. Pharmacol.*, 1990, 39, 1743 .
- M. Jay in *The Flavonoids-Advances in Research since 1986*, ed. J.B. Harborne, Chapman and Hall, London, 1994, 57.
- H. Kumamoto, Y. Matsubara, Y. Iizuka, K. Okamoto and K. Yokoi, *Agric. Biol. Chem.*, 1986, 50, 781.
- Y. Matsubara, H. Kumamoto, A. Sawabe, Y. Iizuka and K. Okamoto, *Yoshishu*, 1985, 27, 702.
- M.A. Castiglione-Morelli, F. Lejl, F. Naider, M. Tallon, T. Tancredi and P.A. Temussi, *J. Med. Chem.*, 1990, 33, 14, and refs. cited therein.
- Arnoldi, A. Bassoli, L. Merlini and E. Ragg, *J. Chem. Soc., Perkin Trans. II*, 1991, 1399 .
- G.E. du Bois and R.A. Stephenson, *J. Agric. Food Chem.*, 1982, 30, 676 .
- E. Joubert, Ph.D. Thesis, University of Stellenbosch, South Africa, 1994 .
- W.E. Hillis and T. Inoue, *Phytochemistry*, 1967, 6, 59 .
- R.M. Horowitz in *Plant Flavonoids in Biology and Medicine*, eds. V. Cody, E. Middleton and J.B. Harborne, A.R. Liss, New York, 1986, 163 .
- R.Y. Wong and R.M. Horowitz, *J. Chem. Soc., Perkin Trans. 1*, 1986, 843 .
- R. Pendse, A.V.R. Rao and K. Venkataraman, *Phytochemistry*, 1973, 12, 2033 .
- T. Tanaka, Y. Orii, G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, 1993, 412, 1232 .
- L.J. Porter and R.W. Hemingway in *Natural Products of Woody Plants II*, ed. J.W. Rowe, Springer-Verlag, Berlin, 1989, 988, and refs. cited therein.
- L. Chalker-Scott and R.L. Kraemer in *Chemistry and Significance of Condensed Tannins*, eds. R.W. Hemingway and J.J. Karchesy, Plenum Press, New York, 1989, 345 .
- F. Petereit, H. Kolodziej and A. Nahrstedt, *Phytochemistry*, 1991, 30, 981 .
- N.R. Farnsworth, O. Akerele, A.S. Bingel, D.D. Soejarto and Z. Guo, *Bull. World Health Organ.*, 1985, 63, 965 .

- D. Lambusta, G. Nicolosi, A. Patti and M. Piattelli, *Synthesis*, 1993, 1155 .
- P. Dittrich and A. Korak, *Phytochemistry*, 1984, 23, 65 .
- T. Hudlicky, J.D. Price, F. Rulin and T. Tsunoda, *J. Am. Chem. Soc.*, 1990, 112, 9439 .
- M. Mandel and T. Hudlicky, *J. Chem. Soc., Perkin Trans. 1*, 1993, 741 .
- D.H.R. Bartonk, P. Dalko and S.D. Gero, *Tetrahedron Letters*, 1991, 32, 2471 .
- D.C. Billington, *Chem. Soc. Rev.*, 1989, 18, 83 .
- M.J. Berridge, *Nature*, 1993, 361, 315 .
- C.R. Narayanan, D.D. Yoshi, A.M. Mujumdar and V.V. Chekne, *Current Science*, 1987, 56, 139 .
- D.T. Hurst and *An Introduction to the Chemistry and Biochemistry of Pyrimidines, Purines and Pteridines*, John Wiley and Sons, Chichester, 1980, 104, 219 .
- D.M.O. Becroft, *New. Engl. J. Med.*, 1984, 310, 133 .
- E. Haslam, *Shikimic Acid, Metabolism and Metabolites*, John Wiley and Sons, Chichester, 1993, 77, 85, 160, 161, 162, 168-169, 172-173 .
- D.G. Roux and D. Ferreira, *Phytochemistry*, 1974, 13, 2039 .
- R.J. Yu and E.J. van Scott, *EP 0 413 528 A1*, 1991 .
- M.J. Thomas, *Crit. Rev. Food Sci. Nutr.*, 1995, 35, 21 .
- Halliwell, J.M.C. Gutteridge and C.E. Cross, *J. Lab. Clin. Med.*, 1992, 199, 598 .
- M. Namiki, *Crit. Rev. Food Sci. Nutr.*, 1990, 29, 273 .
- Halliwell, *FASEB J.* 1987, 1, 358 .
- Halliwell, M.A. Murcia, S. Chirico and O.I. Aruoma, *Crit. Rev. Food Sci. Nutr.*, 1995, 35, 7
- R.S. Sohal, L. Arnold and W.C. Orr, *Drosophila melanogaster. Mech. Ageing Dev.*, 1990, 56, 223 .
- M.N.H. Golden and D. Ramdath, *Proc. Nutr. Soc.*, 1987, 46, 53 .
- W.L. Porter in *Autoxidation in Food and Biological System*, eds. M.G. Simic and M. Karel, Plenum Press, London, 1990, 295 .
- P. Bermond in *Food Antioxidants*, ed. B.J.F. Hudson, Elsevier Applied Science, London, 1990, 193 .
- L.R. Dugan in *Autoxidation in Food and Biological System*, eds. M.G. Simic and M. Karel, Plenum Press, London, 1990, 261 .
- E.A.H. Roberts and R.F. Smith, *J. Sci. Food Agric.*, 1963, 14, 689 .
- D.E. Pratt and B.J.F. Hudson in *Food Antioxidants*, ed. B.J.F. Hudson, Elsevier Applied Science, London, 1990, 17.1
- S.R. Husain, J. Cillard and P. Cillard, *Phytochemistry*, 1987, 26, 2489 .
- J. Torel, J. Cillard and P. Cillard, *Phytochemistry*, 1986, 25, 383 .
- Y. Sorata, U. Takahama and M. Kimura, *Biochim. Biophys. Acta*, 1984, 799, 313 .
- M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, E.J.M. Feskens and D. Kromhout, *Voeding*, 1994, 55, 23 .
- M.B. Korycka-Dahl and T. Richardson, *Crit. Rev. in Food Sci. and Nutr.*, 1978, 10, 209 .
- Tournaire, M. Hocquaux, I. Beck, E. Oliveros and M-T. Maurette, *Tetrahedron*, 1994, 50, 9303 .