Antioxidant scavenging potential of South African export herbal teas

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The flavonoid content as well as the superoxide anion and hydroxyl radical scavenging activities of water extracts of rooibos (*Aspalathus linearis* (Burm. f.) R. Dahlg.), honeybush (*Cyclopia intermedia* E. Mey.) and roselle (*Hibiscus sabdariffa* L.), three South African herbal teas which are commercial products exported world-wide, were investigated. The results indicated that the water extracts of rooibos exhibited the best scavenging activity for both the hydroxyl radical and superoxide anion followed by honeybush and then roselle. This seems to be due to the total flavonoid concentration, which was the highest in rooibos and lowest in roselle.

Next to water, tea is the most popular beverage in the world (Kazi et al. 2002). It is thus not surprising that the antioxidant properties of green, black, oolong and bitter tea have been studied so extensively (Chen et al. 2002, Zhu et al. 2002). Teas exported from South Africa include rooibos (*Aspalathus linearis* (Burm. f.) R. Dahlg.), honeybush (*Cyclopia intermedia* E. Mey.) and roselle (*Hibiscus sabdariffa* L.). Rooibos which means ‘red bush’ in Afrikaans has been enjoyed as both a beverage and a health tonic by the local South African population for centuries. It has been found to help with stomach cramps, reduce stress, relieve babies from colic, cure insomnia, decrease the aging process and many more (Snyckers and Salemi 1974). Externally rooibos is used as treatment of a variety of skin problems including insect stings, sunburn, eczema and rashes (Van Wyk et al. 2000). Honeybush tea is known to calm the central nervous system, ease constipation and stimulate appetite. It has also been used as an expectorant in chronic catarrh and pulmonary disease (Watt and Breyer-Brandwijk 1962). In North Africa and the Near East roselle is called *carcadè* and is known by this name in the pharmaceutical and food-flavouring trades in Europe (Morton 1987). The dried calyxes are used for stimulating appetite, soothing sore throats, laryngitis and tonsillitis, relieving coughs, dryness of the lungs and digestive upsets (Morton 1987).

It has been believed for centuries that teas, especially green tea, has beneficial health properties. Epidemiological studies have shown that the consumption of tea is related to a decrease in the occurrence of cancer in individuals who drink tea regularly (Worthy 1991, Hirose et al. 1993, Mukhtar et al. 1994, Weisburger and Chung 2002). The increasing interest in the health benefits of tea has led to their inclusion in dietary supplements (Higdon and Frei 2003). The health benefits have been ascribed to the high levels of polyphenols, although the mechanism by which they exert their effects is still uncertain (Kuroda and Hara 1999, Yang 1999, Mukhtar and Ahmad 2000, Riemersma et al. 2001). In this study the phenolic content and the superoxide anion and hydroxyl radical scavenging activities of water extracts of rooibos, honeybush and roselle, three South African teas which are commercial products exported around the world, were investigated.

Roselle, rooibos and honeybush were a gift from Dr M Smith of Agribusiness in Sustainable Natural African Plant Products. One gram of grounded plant material was added to 20ml deionised water and the infusion boiled for 15min. The extracts were allowed to cool, centrifuged and the supernatants filtered through 0.22µm filters and the yields determined.

Flavonoid concentration was determined according to Nieva Moreno et al. (2000) and calculated using quercetin as standard (Park et al. 1997). Hydroxyl radical scavenging activity was determined by luminescence. Phenol red free Hanks Balanced Salt Solution (pH 7.4), luminol (110µM), horseradish peroxidase (0.44µM ml⁻¹) and varying concentrations of the teas, total volume 900µl, was incubated at 37°C for 10min. One hundred µl of hydrogen peroxide (20µM) was added by automated injection with the dispenser controller and luminescence (mV) recorded for 6s. The con-
trol sample received no tea extract. Relative peak heights were used to calculate the activity of the test sample in relation to the corresponding controls. Superoxide anion scavenging activity was determined similarly. Luminol and horse-radish peroxidase were replaced with lucigenin (220µM) and xanthine (110µM). Xanthine oxidase (260mU mℓ⁻¹) was added by automated injection and luminescence (mV) recorded for 28min.

The statistical analyses were performed using STATISTIX. A parametric AOV procedure was performed upon ranking of the data, followed by the Fisher LSD Test. Two group comparisons were performed with the Wilcoxon Ranked Sums Test or with the Student’s t-test, depending on the distribution of the data.

The yield for rooibos, honeybush and roselle was 0.79%, 0.40% and 1.96%, respectively. Table 1 shows the flavonoid concentration, hydroxyl radical and superoxide anion scavenging properties of rooibos, honeybush and roselle teas. Flavonoid content and scavenging activities of the tea groups were compared with AOV following ranking of the data. All three means were significantly different from one another. Rooibos tea was significantly superior to the other two teas tested regarding both the scavenging of the superoxide anion as well as the products of the HRP/H₂O₂ system. The flavonoid content of rooibos was the highest, followed by that of honeybush and then roselle.

Antioxidant activities have been documented for vitamins, phenolics and tannins (McCune and Johns 2002). Phenolics, in particular flavonoids have been reported to be efficient antioxidants (Hanasaki et al. 1994, Cao et al. 1997) and have gained interest because of their broad pharmacological activity (Di Carlo et al. 1999). Teas are a rich source of flavonoids and phenolic acids (Hertog et al. 1997a). The calyces of Hibiscus sabdariffa, contain the flavonoids gossypetine, hibiscetin and sabdarentine (Dafallah and al-Mustafa 1996) and anthocyanins. Rooibos contains a complex mixture of polyphenolic compounds, of which aspalathin is unique to this plant, as well as the flavonol, quercetin, the flavone luteolin and five flavonoid glycosides (Rabe et al. 1994). Honeybush contains the phenolic compounds: mangiferin, hesperitin, isosakuranetin and luteolin (Rabe et al. 1994, De Nyschen et al. 1996, Von Gadow et al. 1997a). The latter is the most important flavonoid in honeybush whereas the former three are chemical markers of the genus Cyclopia (De Nyschen et al. 1996). The flavonoid concentration of 38.94% for rooibos obtained in this study confirms results reported by Von Gadow et al. (1997b). No comparative values for the flavonoid concentration of roselle or honeybush could be obtained from the literature.

Ethanol extracts of the dried flowers of Hibiscus sabdariffa have been shown to quench the 1,1-diphenyl-2-picrylhydrazyl derived free radicals as well as superoxide derived from xanthine/xanthine oxidase (Tseng et al. 1997). The anthocyanins; delphinidin 3-sambubioside and cyaniding 3-sambubioside are responsible for 51% of the total antioxidant capacity of roselle petal extract (Bartosz 2003).

It is proposed that the health aspects of tea are linked to its phenolic compounds such as the flavonoids and is associated with antioxidant activity (Niwa and Miyachi 1986, Yang 1999, Mukhtar and Ahmad 2000, Riemersma et al. 2001, Kamara et al. 2003). Flavonoid intake can range between 50 and 800mg day⁻¹ depending on the consumption of vegetables and fruit and of specific beverages such as red wine, tea and unfiltered beer (Larson 1988). Tea contains approximately 200mg of total phenolic components per cup (Pietta 2000). From our results it would appear that the higher hydrogen donating and O₂⁻ scavenging abilities of rooibos compared to honeybush and roselle respectively, was due to the corresponding flavonoid concentrations.

The most common oxidants are the superoxide and hydroxyl radicals and hydrogen peroxide which are referred to as reactive oxygen species (Halliwell 1996). Reactive oxygen species such as the superoxide anion and hydroxyl radical have been implicated in the pathophysiology of various clinical disorders including cancer as consumption of tea has been shown to reduce the risk of various cancers (Worthy 1991, Hirose et al. 1993, Mukhtar et al. 1994). Rooibos tea, honeybush tea and roselle have been shown to contain antimutagenic activity possibly due to its antioxidant properties (Chewonarin et al. 1999, Marnewick et al. 2000). The anthocyanins found in hibiscus are anti-inflammatory and function by coating the surface of cell membranes and protect them from enzymatic and free radical damage (Dafallah and al-Mustafa 1996). Analysis of rooibos extracts have shown that the free radical scavenging activities are

Table 1: Total flavonoid concentration, hydroxyl radical and superoxide anion scavenging properties of water extracts of the teas tested. P values were determined using the Wilcoxon rank Sum Test for the flavonoid content and the Student’s t-test for both the oxidant scavenging activities

<table>
<thead>
<tr>
<th>Tea</th>
<th>Flavonoid content (%)</th>
<th>OH scavenging* (%)</th>
<th>O₂⁻ scavenging* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 8/group</td>
<td>n = 3/group</td>
<td>n = 3/group</td>
</tr>
<tr>
<td>Rooibos (A)</td>
<td>38.94 ± 2.47</td>
<td>6.66 ± 1.39</td>
<td>39.37 ± 2.72</td>
</tr>
<tr>
<td>Honeybush (B)</td>
<td>27.77 ± 1.07</td>
<td>33.06 ± 4.90</td>
<td>61.83 ± 1.68</td>
</tr>
<tr>
<td>Roselle (C)</td>
<td>24.90 ± 0.97</td>
<td>84.27 ± 8.01</td>
<td>89.29 ± 8.63</td>
</tr>
<tr>
<td>P value</td>
<td>A/B: 0.0009</td>
<td>0.0009</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>A/C: 0.0009</td>
<td>0.0250</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>B/C: 0.0009</td>
<td>0.0496</td>
<td>0.0277</td>
</tr>
</tbody>
</table>

* Results are expressed at peak heights relative to the corresponding controls for triplicate experiments at a concentration of 1.25µg mℓ⁻¹ for the hydroxyl radical and 5.0µg mℓ⁻¹ for the superoxide anion scavenging properties, respectively.
possibly due to an enzyme that mimics the enzyme superoxide dismutase (Yoshikawa et al. 1990, Marnewick et al. 2000, Standley et al. 2001, Zhu et al. 2002).

This study confirmed the results obtained by Lindsey et al. (2002) that the South African teas have antioxidant activity in the following range: rooibos > honeybush > roselle. However, this is the first study to compare this activity to the flavonoid content. Similar to the antioxidant activity it was found that rooibos was superior to the other teas tested regarding flavonoid content, followed by honeybush, with roselle having the lowest flavonoid content.

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