Evaluation of the content of polyphenolic-based antioxidant compounds of different flavored and non-flavored Rooibos (Aspalathus linearis) infusions

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Abstract: The aim of the study was to comparatively evaluate the content of flavonoids and tannins, the antioxidant activity as determined by total phenolics, and sensory analysis of Rooibos infusions originating from twelve different flavored and non-flavored commercial samples. The obtained results showed higher mean values of total phenolics content in flavored samples compared to the other ones due to the contribution of additional components (vanilla, cinnamon, orange, lemon, and caramel). High content of total flavonoids was found in all investigated samples of Rooibos tea without significant differences between the two groups. There was a great variability of the content of total condensed tannins with a twofold increase in flavored samples. Regarding the sensory attributes, the average scores for overall aspect, color, aroma, flavor and persistence attributes were slightly higher for non-flavored Rooibos infusions than those assigned to flavored ones. Among all investigated samples, higher scores were assigned for color and strength attributes, while lower scores were given to the overall aspect.

The results show that Rooibos tea can be a valuable additional source of dietary antioxidants of polyphenolic structure.

Key words: antioxidant; flavonoids; phenolics; Rooibos tea; sensory evaluation; tannins.

Introduction

As most research studies agree on the positive influence of a polyphenolic-rich diet on the human health, the consumption of foods abundant in such compounds – mainly fruits and vegetables – increased significantly. The diet rich in antioxidant compounds might additionally include beverages other foods, such as herbal tea, specific teas and wine.

Herbal teas have been for long considered an essential source of phytopharmaceuticals of great significance in traditional medicine for human health (Wichtl, 2004). Rooibos or red bush tea is a herbal tea from the African plant Aspalathus linearis (Fabaceae, Tribe Crotalariaceae). Either in the form of fermented or un-fermented/green, it is consumed as a healthy beverage, which recently became more popular worldwide due to the research studies reporting its biological properties and benefits. The composition of Rooibos tea is different from the green/black tea of Camellia sinensis. It contains various flavonoids with the predominant unique flavonoid called aspalathin, tannins, phenolic acids, stilbenoids, lignans, macro- and micronutrients, and is caffeine-free (Rusinek-Prystupa et al., 2016). The particular polyphenolics composition of un-fermented tea changes in the traditional fermented tea. Thus, aspalathin is oxidized to other compounds, iso-orientin and orientin (Krafczyk et al., 2009).

The high content of compounds of polyphenolic structure is considered to mainly contribute to the high antioxidant activity of Rooibos. The in vitro and in vivo assays showed the potential of this herbal tea to protect cells from oxidative damage (Marnewick, 2014). Other reported biological properties of Rooibos are anti-inflammatory, anti-carcinogenic, hypoglycemic, anti-hyperlipidemic, anti-adipogenetic (Joubert and Beer, 2011).

Due to the bioactivity of Rooibos extracts, there is a great potential for research on extraction of specific valuable compounds, mechanism of action, and for finding new applications, such as cosmetics, dietary supplements, functional foods and beverages.

The aim of the present paper was to evaluate, compare and discuss the content of flavonoids and tannins, and the antioxidant activity as determined by total phenolics, of Rooibos infusions of 12 different samples cultivated in various locations and available on the Romanian market. Sensory analysis of Rooibos infusions was also performed.
Materials and Methods
Sample preparation
A number of 12 samples of various types of Rooibos tea in bags, produced by different manufacturers, was investigated. Six samples were pure Rooibos tea, while the other six were differently flavored Rooibos tea. The samples listed in Table 1 were collected at random from the Romanian local market and prepared in water as indicated on the label by each manufacturer.

Table 1. Characteristics of investigated Rooibos tea samples.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Product name / type</th>
<th>Method of preparation from bag tea, 5 min</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mass (g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water (mL)</td>
<td></td>
</tr>
<tr>
<td>Non-flavored Rooibos tea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Lord Nelson Rooibos tea</td>
<td>2.00</td>
<td>5.872</td>
</tr>
<tr>
<td>2</td>
<td>Dukat Rooibos tea</td>
<td>2.00</td>
<td>5.680</td>
</tr>
<tr>
<td>3</td>
<td>Sonnentor Rooibos tea</td>
<td>1.09</td>
<td>6.411</td>
</tr>
<tr>
<td>4</td>
<td>Fares Rooibos tea</td>
<td>1.50</td>
<td>5.826</td>
</tr>
<tr>
<td>5</td>
<td>AdNatura Rooibos tea</td>
<td>1.00</td>
<td>6.410</td>
</tr>
<tr>
<td>6</td>
<td>Rioba Rooibos tea</td>
<td>2.00</td>
<td>5.251</td>
</tr>
<tr>
<td>Flavored Rooibos tea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Lord Nelson Rooibos tea vanilla flavor</td>
<td>2.00</td>
<td>5.683</td>
</tr>
<tr>
<td>8</td>
<td>Dukat Rooibos tea orange and cinnamon flavor</td>
<td>2.00</td>
<td>4.707</td>
</tr>
<tr>
<td>9</td>
<td>Sonnentor Rooibos tea orange flavor</td>
<td>1.65</td>
<td>5.611</td>
</tr>
<tr>
<td>10</td>
<td>Fares Rooibos tea caramel flavor</td>
<td>1.50</td>
<td>5.368</td>
</tr>
<tr>
<td>11</td>
<td>Evolet Rooibos tea lemon flavor</td>
<td>1.50</td>
<td>4.747</td>
</tr>
<tr>
<td>12</td>
<td>Bio3 Rooibos tea orange flavor</td>
<td>1.50</td>
<td>3.877</td>
</tr>
</tbody>
</table>

Determination of total phenolics
The total phenolics content of Rooibos infusions was determined by the Follin-Ciocalteu assay (Singleton and Rossi, 1965). Values were calculated based on a standard calibration curve of gallic acid (GAE) and expressed as mg GAE 100g⁻¹ DM.

Determination of total flavonoids
The total flavonoids content of Rooibos infusions was determined by the colorimetric method (Bag et al., 2015). Values were calculated based on a standard calibration curve of quercetin and expressed as mg quercetin 100g⁻¹ DM.

Determination of total condensed tannins
The tannin content of Rooibos infusions was determined by the method of Broadhurst et al. (Broadhurst and Jones, 1978). Values were calculated based on a standard calibration curve of catechin and expressed as mg catechin 100g⁻¹ DM.

Sensory evaluation
The following sensory attributes were analyzed during the sensory test of tea samples prepared according to manufacturer information: overall aspect, color, texture/body, strength, aroma, flavor and persistence of taste in mouth. A trained panel of 10 panelists was engaged for the sensory analysis. Panelists were provided with an evaluation chart for the record of their assessment. The 5 point score card was used to point out the perceived intensities of the seven investigated attributes.
Statistical analysis
All measurements were performed in duplicate. Results were calculated and expressed as mean ± standard deviation of triplicate determination.

Results and Discussion
The present study illustrates the results of the evaluation of several key antioxidant compounds of polyphenolic structure (flavonoids, phenolics, tannins) of Rooibos tea samples. The content of such phytochemicals was determined directly in the prepared infusions, as described by each manufacturer, while most other authors used extracts of *Aspalathus linearis*, usually involving 30 minutes as extraction time and several steps of high-temperature extraction (hot water) or organic solvents (ethanol-water, acetonitrile-water).

The mean value of the content of total phenolics determined by Folin-Ciocalteu method for the selected 12 samples of Rooibos tea was found 2269.68±399.54 mg GAE 100g⁻¹ DM. The results are shown in Figure 1.

![Figure 1. Total phenolics content of Rooibos tea samples (samples 1-12, as described in Table 1).](image)

By studying the difference between the two groups of samples, flavored and non-flavored Rooibos tea, respectively, we found higher values for flavored samples (2344.72±428.52 mg GAE 100g⁻¹ DM) compared to the other ones (2194.63±392.49 mg GAE 100g⁻¹ DM), with the highest value for the Fares Rooibos tea with caramel flavor (2997.39 mg GAE 100g⁻¹ DM) and the lowest for the Sonnentor Rooibos tea with orange flavor (1903.84 mg GAE 100g⁻¹ DM). The greater level of phenolics might be explained by the contribution of phenolic compounds from the other added teas or aroma in the investigated samples. Other authors reported higher amounts of phenolic acids in caramel flavored Rooibos tea, such as Lord Nelson with caramel (0.88% phenolic acids) and lower in Taheebo and PackerBest with orange (0.23% phenolic acids) (Rusinek-Prystupa et al., 2016). Among the studied pure Rooibos tea samples, the highest value was found for Rioba Rooibos tea (2761.73 mg GAE 100g⁻¹ DM).

The main phenolic acids reported to be present in *Aspalathus linearis* tea are caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, protocatechuic, syringic and vanillic acids (Cabooter et al., 2011).

Regarding the content of total flavonoids, the mean value for the 12 investigated samples of Rooibos tea was found 4770.54±1023.76 mg quercetin 100g⁻¹ DM. The results are shown in Figure 2.
Concerning the two groups of samples, flavored and non-flavored Rooibos tea, no significant differences were found for the mean values of total flavonoids. The highest content was found for the Rioba Rooibos tea (6524.63 mg quercetin 100g\(^{-1}\) DM). Among flavored samples, the Fares Rooibos tea with caramel flavor showed great amounts of flavonoids (5996.78 mg quercetin 100g\(^{-1}\) DM), while the Sonnentor Rooibos tea with orange flavor showed the lowest level (3621.90 mg quercetin 100g\(^{-1}\) DM).

It has been reported that quercetin, rutin, luteolin, chrysoeriol, nothofagin, orientin, iso-orientin, vitexin, iso-vitexin and aspalanthin are the main flavonoids found in Rooibos tea, the latter being a dihydrochalcone unique to *Aspalathus linearis* (Cabooter at al., 2011).

The average content of total condensed tannins in the 12 investigated samples of Rooibos tea, as measured by the spectrophotometrical vanillin assay, was 890.69±491.20 mg catechin 100g\(^{-1}\) DM and ranged from 487.80 mg catechin 100g\(^{-1}\) DM (AdNatura Rooibos tea) to 2127.93 mg catechin 100g\(^{-1}\) DM (Bio3 Rooibos tea with orange flavor). The results are shown in Figure 3.
Regarding the two groups of samples, simple and flavored Rooibos tea, we found higher values for flavored samples (1174.43±568.27 mg catechin 100g⁻¹ DM) almost double compared to non-flavored ones (606.96±121.15 mg catechin 100g⁻¹ DM). Among the investigated simple Rooibos tea samples, the highest value was found for Rioba Rooibos tea. These results are in agreement with other reported studies demonstrating the low content of tannins in pure Rooibos tea and greater amounts in flavored Rooibos tea (Joubert and Beer, 2011). Polish authors reported a slightly lower average content of tannins extracted from tea bags of 3.67±2.07 g kg⁻¹ DM, and of tea leaves of 4.95±1.35 g kg⁻¹ DM, as measured by a different analytical method (mass titration) (Rusinek-Prystupa et al.). Higher amounts of tannins in Rooibos tea after boiling 30 minutes (4.4% as gallic acid) were previously reported (Morton, 1983).

The comparison of the content of bioactive compounds of polyphenolic structure might be a difficult task, as different extractive methodologies, analytical quantification methods and final calculation/expression of the results have been used in published papers. Besides these, some other important factors influence the levels of phenolic-based compounds, such as genetic properties, origin of material, processing of leaves and stems (fermented and un-fermented) and preparation of the extract or infusion (Dmowski et al., 2011; Joubert et al., 2008).

We found a strong positive correlation at p<0.01 between the content of total phenolics and that of total flavonoids, and no significant correlations between the other values, as described in Table 2. Considering the determined pH of the prepared Rooibos tea infusions (Table 1), a moderate negative correlation (R=- 0.703) was found between the content of tannins and the pH of the Rooibos tea infusion at p<0.05.

Table 2. Pearson correlation coefficient between the content of bioactive compounds in Rooibos tea samples.

<table>
<thead>
<tr>
<th></th>
<th>Total phenolics</th>
<th>Total flavonoids</th>
<th>Total tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics</td>
<td>0.9398</td>
<td>0.2769</td>
<td></td>
</tr>
<tr>
<td>Total flavonoids</td>
<td>0.9398</td>
<td>0.1221</td>
<td></td>
</tr>
<tr>
<td>Total tannins</td>
<td>0.2769</td>
<td>0.1221</td>
<td></td>
</tr>
</tbody>
</table>

The origin of the Rooibos tea greatly influences the content of levels of bioactive compounds.

**Sensory evaluation**

The results regarding the recorded scores of seven sensory attributes (overall aspect, color, texture/body, strength, aroma, flavor and persistence of taste in mouth) of Rooibos infusions are presented in Figure 4.

![Spider plot for 12 commercial Rooibos infusions (1-6 non-flavored tea samples and 7-12 flavored tea samples) characterized by different sensory attributes.](image)

The average scores for overall aspect, color, aroma, flavor and persistence attributes were slightly higher for un-flavored Rooibos infusions than those assigned to flavored ones. Among all investigated samples, higher scores were assigned for color and strength attributes, while lower scores were given to the overall aspect. With regard to sensory quality of Rooibos infusions from non-flavored tea samples, other studies reported a mixture of characteristics, such as honey, woody and herbal-floral flavors and a sweet taste (Koch et al., 2012).

**Conclusions**

Teas containing flavored and non-flavored Rooibos can be a significant source of antioxidant compounds, such as phenolics, flavonoids and tannins. Higher mean values of total phenolics content were found in flavored samples...
(2344.72±428.52 mg GAE 100g⁻¹ DM) compared to the other ones (2194.63±392.49 mg GAE 100g⁻¹ DM), due to the contribution of additional components (vanilla, cinnamon, orange, lemon, caramel). The content of total flavonoids for the 12 investigated samples of Rooibos tea showed a mean value of 4770.54±1023.76 mg quercetin 100g⁻¹ DM to 2127.93 mg catechin 100g⁻¹ DM with a twofold increase in flavored samples.

Regarding the sensory attributes, the average scores for overall aspect, color, aroma, flavor and persistence were slightly higher for non-flavored Rooibos infusions than those assigned to flavored ones. Among all investigated samples, higher scores were assigned for color and strength attributes, while lower scores were given to the overall aspect.

The results of the present investigation showed that Rooibos tea can be a beneficial additional source of dietary antioxidants.

References