

Antioxidant capacity of herbal teas from Bulgarian market

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Abstract

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The antioxidant capacity of numerous plant species with medical potential, as well as the tea plant (*Camellia sinensis* L.) have been extensively studied. On the other hand, such data for herbal tea blends, taken directly from the market are scarce.

The aim of our study was to evaluate the antioxidant capacity and phenol content of tea and herbal tea blends available on Bulgarian market and to assess *Haberlea rhodopensis* leaves as a potential ingredient of herbal teas. We applied two methods to evaluate the antioxidant capacity – the Ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays, after a two step procedure of processing and extraction of water-soluble and lipo-soluble antioxidants.

We did not find a direct relationship between the content of phenols and the antioxidant capacity of the studied herbal teas. The commercial samples of *Camellia sinensis* L. (North Vietnam cultivation, Tikanium, Green tea) had the highest antioxidant capacity. According to TEAC and FRAP assay Common St John's wort, Oregano, and Garden sage showed antioxidant capacity close to that of Green tea.

Maximum antioxidant capacity and, hence maximal health benefits could be obtained from ca. 30 min infusions of Roselle tea and especially *Haberlea* leaves. This makes cultivated *Haberlea rhodopensis* a valuable potential ingredient for new blends of herbal tea with high antioxidant capacity.

Keywords: Antioxidants; FRAP; TEAC; TAC; phenols; herbal teas; *Haberlea rhodopensis*

Abbreviations: TAC – Total antioxidant capacity; TEAC – Trolox equivalent antioxidant capacity; FRAP – Ferric reducing antioxidant power

Introduction

Plants have been used in traditional medicine as a source of natural bioactive compounds from the very beginning of human civilization. In recent years, intensive studies have been focused on the antioxidative properties of various plant species (Du Toit et al., 2001; Zheng & Wang, 2001; Trouillas et al., 2003; Cai et al., 2004; Katalinic et al., 2006; Cleverdon et al., 2018). In most cases, however, the studies are not performed with commercial blends or products taken from

the market. The exceptions are related to commercial products derived from the tea plant species (*Camellia sinensis* L.) since this type of drink is the most consumed beverage, second only to water (McKay & Blumberg, 2002). Tea is one of the richest sources of antioxidants and as such, has numerous beneficial health effects (Su et al., 2007). The flavonoids present in tea have been shown to have strong antioxidant and metal-chelating properties *in vitro* and, hence, may be expected to protect cells and tissues against free oxygen radicals. Tea polyphenols, for example, reduce the risk of heart

diseases and cancer in humans (Crespy & Williamson, 2004; Cao et al., 2019).

The use of natural herbs for hot drinks (herbal teas) in Bulgaria is very common and traditional. A wide variety of commercial blends is available on the Bulgarian market.

Haberlea rhodopensis is a Balkan endemic plant species. It belongs to the small group of so-called “resurrection species” – plants that are able to survive long periods of full desiccation and to recover fast upon re-watering. These species are under intensive studies because of their potential as a source for useful genes to improve the drought tolerance of crops (e.g. Dinakar et al., 2012; Liu et al. 2019). Additionally, it is considered as a source of numerous compounds with antioxidant properties that are of potential use in health care, cosmetics, and food (Gechev et al., 2014, Moyankova et al., 2014; Moyankova & Djilianov, 2016; Todorova & Atanassov, 2016).

The aim of our study was to evaluate the antioxidant capacity and phenols of tea and herbal tea blends available on Bulgarian market. The potential of *Haberlea rhodopensis* leaf samples as a plant material for herbal teas was compared with existing commercial products.

Materials and Methods

Sampling

Commercial blended tea-bags of the following teas and herbal teas were used: Green tea – North Vietnam cultivation (*Camellia sinensis* L.), Green tea (*Camellia sinensis* L.), Tikuanym tea – Oolong tea (*Camellia sinensis* L.), Oregano (*Origanum vulgare* L.), Peppermint (*Mentha arvensis* L.), Black dogwood (*Frangula alnus* Mill.), Garden sage (*Salvia officinalis* L.), Common St John’s wort (*Hypericum perforatum* L.), Mountain tea (*Sideritis scardic* Griseb.), Rooibos tea (*Aspalathus linearis*) and Roselle tea (*Hibiscus sabdarifa* L.).

Leaves of *Haberlea rhodopensis* were air-dried at room temperature and mixed randomly to establish standard “tea-bags” samples, similar to commercial blends of tea and herbal tea. The leaves were detached from potted plants, routinely cultivated for one year at the greenhouse of AgroBioInstitute after *in vitro* regeneration and propagation as described earlier (Djilianov et al., 2005). Samples of 2 g air-dried leaves (*H. rhodopensis*) or filter tea bags contents (commercial blend teas) were ground with pestle and mortar in 0.1% trichloroacetic acid. The homogenates were centrifuged at 15 000 g for 30 min and the resulting supernatants were analyzed for TEAC and FRAP assays, after a two step procedure of processing and extraction of water-soluble and lipo-soluble (acetone extract) antioxidants (Kerchev & Ivanov, 2008).

Total antioxidant capacity evaluation

The TEAC assay is based on the ability of antioxidant molecules to quench the long-lived ABTS^{•+} radical (Pellegrini et al., 1999). A stable stock solution of ABTS^{•+} was produced by reacting an aqueous or ethanol solution of ABTS with potassium persulfate and allowing the mixture to stand at dark at room temperature for 12–16 h before use. Plant samples (20 µl) were added to 200 µl of the reagent in the wells of a 96-well microplate. The absorption was read at 734 nm after 30 min. The results were calculated according to standard curves prepared with known concentrations of Trolox (water-soluble vitamin E analog) and were expressed as µmol Trolox.g⁻¹.

The FRAP assay is based on the reduction of the Fe³⁺TPTZ complex to the ferrous form at low pH (Benzie & Strain, 1996). Freshly prepared FRAP reagent (0.2 ml) (with ethanol or distilled water) was added to 20 µl of the diluted samples in the wells of a 96-well microplate and the absorbance at 593 nm was recorded after a 30 min incubation at 37°C. The results were calculated according to standard curves prepared with known concentrations of Fe²⁺, and were expressed as µmol Fe²⁺.g⁻¹.

Total water-soluble phenols

Phenols were determined with the Folin-Ciocalteu reagent supplemented with sodium carbonate and the absorbance was read at 725 nm according to Swain & Goldstein (1964) with minor modifications. Gallic acid was used as a reference standard. The results are presented as µmol.g⁻¹.

Aqueous tea infusions

To prepare tea infusions from *H. rhodopensis* and roselle teas, 2 g of the air-dried herb (leaves) were soaked in 200 ml of boiling distilled water for 5 min and for a longer period (ca. 30 min) of infusion (commonly used domestic methods of preparation). Then, the obtained beverages were investigated for TEAC and FRAP.

All spectrophotometric measurements were made with a microplate reader (Multiscan Spectrum, Thermo Electron Corporation). The results represent mean values from two independent repetitions ± standard deviations.

Results and Discussion

Total antioxidant capacity (TAC) is a parameter that takes into account all the synergistic and cumulative interactions between known and unknown antioxidants present in a sample (Kerchev & Ivanov, 2008). It reflects both hydrophilic and lipophilic antioxidant properties, allowing the activity of both types of antioxidants to be estimated in one

sample. There are numerous methods for TAC evaluation in different systems – e.g. “TRAP” (Total Radical-Trapping Antioxidant Parameter), “TEAC” (Trolox Equivalent Antioxidant Capacity), “ORAC” (Oxygen-Radical Absorbance Capacity), “FRAP” (Ferric/Reducing Antioxidant Power) (Sanchez-Moreno, 2002).

In our studies, we applied TEAC and FRAP. While TEAC assay is based on the antioxidant’s ability to react with ABTS⁺ radical cation generated in the system, FRAP evaluates the reduction of ferric iron (Fe³⁺) to ferrous iron (Fe²⁺) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above that of Fe³⁺/Fe²⁺. The latter assay is also commonly used for routine analysis of single antioxidants and total antioxidant activity of plant extracts, showing the corresponding concentration of electron-donating antioxidants (Gao et al., 2000; Halvorsen et al., 2002; Schlesier et al., 2002).

TEAC assay

A considerable variation in antioxidant activity was observed among the tested tea blends (Table 1). The total antioxidant activity, measured by the TEAC method, ranged from 141.3 to 2485.3 μmol Trolox per two grams of tea (the content of a standard tea bag for a 200 ml cup of tea). The data for the aqueous extracts, expressed in Trolox equivalents per gram of DW, ranged from (50.2 \pm 2.8) $\mu\text{mol}\cdot\text{g}^{-1}$ to (626.1 \pm 16.1) $\mu\text{mol}\cdot\text{g}^{-1}$, and the acetone extract values ranged from (12.1 \pm 2.8) $\mu\text{mol}\cdot\text{g}^{-1}$ to (616.6 \pm 54.8) $\mu\text{mol}\cdot\text{g}^{-1}$. The rank of the same sample for water-soluble and lipo-soluble antioxidant capacity is different.

Green tea (North Vietnam cultivation) showed the highest antioxidant activity, followed by Oolong tea (1510.4 μmol Trolox/tea bag of 2 g). It is important to note that during the preparation of green tea as a commercial product, the fresh tea leaves are steamed and then dried relatively rapidly to avoid enzymatic oxidation. This stops the oxidation of tea leaf catechins catalyzed by the polyphenol oxidase enzyme (Wilson & Clifford, 1992; Abudurehman et al., 2022). Oolong tea is semi-fermented to allow moderate enzymatic oxidation during processing. Although Green and Oolong teas are made from the leaves of the same plant species, *Camellia sinensis* L., various leaf processing technologies result in different chemical compositions, which could explain the lower antioxidant capacity of Oolong tea (Tikuanium tea) compared to Green tea (North Vietnam cultivation).

A positive relationship between the content and structure of phenols and the antioxidant activity of tea samples was demonstrated in other works (e.g. Rice-Evans et al., 1997; Cai et al., 2004; Hussein et al., 2022). Interestingly, Oregano (*Origanum vulgare* L.) showed lower antioxidant potential than Green tea (Tables 1 and 3) but the highest water-soluble phenol content (625.15 \pm 58.81 $\mu\text{mol}\cdot\text{g}^{-1}$) (Table 2) suggesting that the relationship between high antioxidant capacity and total phenol content may not always be direct. Similar results have been reported by other authors (Ivanova et al., 2005).

Relatively moderate antioxidant capacities (values lower than 400 μmol Trolox/tea-bag of 2 g) were determined for the other herbal teas – Mountain tea (323 μmol Trolox/tea-bag of 2 g), Black dogwood (265.4 μmol Trolox/tea-bag of 2 g), Peppermint (166.02 μmol Trolox/tea-bag of 2 g),

Table 1. Antioxidant capacity of teas according to TEAC assay

The data are presented as μmol Trolox $\cdot\text{g}^{-1}$ \pm standard deviation. Total TAC (right column) is calculated as sum of water and lipo-soluble fraction and expressed as μmol Trolox per one cup (200 ml) of tea (2 g). The plant species are arranged according to their TAC in descending order

Plant	Common name	Water-soluble fraction	Rank	Lipo-soluble fraction	Rank	TAC per one cup of tea
<i>Camellia sinensis</i> L.	Green tea (North Vietnam cultivation)	626.1 \pm 16.1	1	616.6 \pm 54.8	1	2485.3
<i>Camellia sinensis</i> L.	Oolong tea (Tikuanium tea)	360.3 \pm 8.8	3	395.0 \pm 42.1	2	1510.4
<i>Origanum vulgare</i> L.	Oregano	426.5 \pm 20.7	2	112.2 \pm 47.1	6	1077.3
<i>Camellia sinensis</i> L.	Green tea	297.1 \pm 3.2	4	240.0 \pm 11.4	3	1074.1
<i>Hypericum perforatum</i> L.	Common St John’s wort	292.1 \pm 12.4	5	145.7 \pm 7.8	4	875.7
<i>Salvia officinalis</i> L.	Garden sage tea	284.2 \pm 12.5	6	136.7 \pm 9.6	5	841.9
<i>Sideritis scardica</i> Griseb.	Mountain tea	103.0 \pm 16.8	8	58.5 \pm 16.0	8	323.0
<i>Haberlea rhodopensis</i> L.	Haberlea	112.9 \pm 2.3	7	44.3 \pm 4.1	9	314.5
<i>Frangula alnus</i> Mill. Alder Buckthorn.	Black dogwood	58.0 \pm 1.8	11	74.7 \pm 2.2	7	265.4
<i>Mentha arvensis</i> L.	Peppermint	59.8 \pm 3.1	10	23.3 \pm 7.1	10	166.2
<i>Hibiscus sabdariffa</i> L.	Roselle	68.9 \pm 11.4	9	12.1 \pm 2.8	12	161.9
<i>Aspalathus linearis</i> L.	Rooibos tea	50.2 \pm 2.8	12	20.4 \pm 5.6	11	141.3

Table 2. Water soluble phenol content in teas. Results are presented as $\mu\text{mol.g}^{-1} \pm \text{S. D.}$

Plant	Common name	Total phenols mean \pm S.D. ($\mu\text{mol.g}^{-1}$)	Rank
<i>Origanum vulgare</i> L.	Oregano	625.15 \pm 58.81	1
<i>Camellia sinensis</i> L.	Green tea (North Vietnam cultivation)	264.45 \pm 31.28	2
<i>Hypericum perforatum</i> L.	Common St John's wort	252.55 \pm 43.61	3
<i>Camellia sinensis</i> L.	Green tea	238.63 \pm 17.04	4
<i>Salvia officinalis</i> L.	Garden sage tea	235.45 \pm 29.21	5
<i>Camellia sinensis</i> L.	Oolong tea (Tikuanium tea)	214.46 \pm 16.53	6
<i>Sideritis scardica</i> Griseb.	Mountain tea	207.80 \pm 34.28	7
<i>Haberlea rhodopensis</i> L.	Haberlea	184.33 \pm 30.64	8
<i>Hibiscus sabdarrifa</i> L.	Roselle	73.23 \pm 10.23	9
<i>Frangula alnus</i> Mill. Alder Buckthorn	Black dogwood	68.47 \pm 10.10	10
<i>Aspalathus linearis</i> L.	Rooibos tea	59.96 \pm 8.82	11
<i>Mentha arvensis</i> L.	Peppermint	50.42 \pm 4.17	12

Roselle tea (161.09 $\mu\text{mol Trolox/tea-bag}$ of 2 g) and Rooibos teas (122.4 $\mu\text{mol Trolox/tea-bag}$ of 2 g). The values of the Haberlea samples were in the same group, with 314.5 $\mu\text{mol Trolox/tea bag}$ of 2 g. The phenol content values were also moderate (Table 2).

The low antioxidant potential of Rooibos tea infusions in our study (Table 1, Table 3) is contrary to other data (von Gadov et al., 1997). On the other hand, Du Toit et al. (2001) measured a considerably lower content of vitamin C in tea from Rooibos in comparison to Green, Oolong, and Peppermint teas. The polyphenol antioxidants identified in Rooibos tea include the flavonoids aspalathin, nothofagin, quercetin, rutin, isoquercitrin, orientin, isoorientin, luteolin, vitexin, isovitexin, and chrysoeriol (Koeppen & Roux, 1966). Green tea contains high levels of catechin epigallocatechin-3-gallate, not

contained in Rooibos. In another study, Ivanova et al. (2005) found lower phenolic content and lower antioxidant activity (by TEAC assay) in Rooibos tea in comparison to other tea samples (e.g. *Hypericum perforatum* L. and *Camellia sinensis* L.). These findings are in agreement with our results that Rooibos tea (59.96 \pm 8.82 $\mu\text{mol.g}^{-1}$) and Peppermint (50.42 \pm 4.71 $\mu\text{mol.g}^{-1}$) were with lowest phenols content.

In most cases, the TEAC and FRAP values of aqueous extracts were higher than those of acetone extracts. Only in Oolong tea (for TEAC assay) and Black dogwood (for TEAC and FRAP assay) the lipo-soluble fraction prevailed. This exception indicates that lipophilic antioxidants (lipophilic phenolics, carotenoids, and vitamin E) are also major contributors to antioxidant activity, which does not exclude a synergistic interaction between water and lipid-soluble antioxidants.

Table 3. Antioxidant capacity of teas according to FRAP assay

The data are presented as $\mu\text{mol Fe}^{2+}.\text{g}^{-1} \pm$ standard deviation. Total TAC (right column) is calculated as sum of water and lipo-soluble fraction and expressed as $\mu\text{mol Trolox per one cup (200 ml)}$ of tea (2 g). The plant species are arranged according to their TAC in descending order

Plant	Common name	Water-soluble fraction	Rank	Lipo-soluble fraction	Rank	TAC per one cup of tea
<i>Camellia sinensis</i> L.	Green tea (North Vietnam cultivation)	721.7 \pm 17.3	1	516.4 \pm 69.5	1	2476.1
<i>Camellia sinensis</i> L.	Oolong tea (Tikuanium tea)	655.3 \pm 27.9	2	273.4 \pm 27.5	3	1857.3
<i>Camellia sinensis</i> L.	Green tea	494.5 \pm 15.1	4	416.5 \pm 15.4	2	1821.9
<i>Origanum vulgare</i> L.	Oregano	481.4 \pm 40.8	5	166.0 \pm 8.1	4	1294.8
<i>Hypericum perforatum</i> L.	Common St John's wort	537.2 \pm 20.9	3	50.8 \pm 14.0	9	1176.0
<i>Salvia officinalis</i> L.	Garden sage	428.8 \pm 21.9	6	90.4 \pm 14.6	6	1038.4
<i>Haberlea rhodopensis</i> L.	Haberlea	421.2 \pm 14.5	7	91.5 \pm 10.3	5	1025.5
<i>Hibiscus sabdarrifa</i> L.	Roselle	167.4 \pm 6.2	8	76.2 \pm 6.3	7	487.1
<i>Sideritis scardica</i> Griseb.	Mountain tea	125.4 \pm 4.1	10	49.0 \pm 24.4	10	348.7
<i>Frangula alnus</i> Mill. Alder Buckthorn.	Black dogwood	35.3 \pm 4.4	12	76.0 \pm 9.7	8	222.6
<i>Mentha arvensis</i> L.	Peppermint	79.0 \pm 2.4	10	26.3 \pm 8.9	11	210.6
<i>Aspalathus linearis</i> L.	Rooibos tea	43.3 \pm 5.6	11	17.9 \pm 2.5	12	122.4

Table 4. Antioxidant capacity of tea infusion according to TEAC and FRAP assay

One tea bag (2 g dried tea leaves) is placing in 200 ml of boiling distillate water for 5 min and for longer period of infusion to complete cooling of tea infusion. Results are presented as μmol Trolox per cup for TEAC assay and as $\mu\text{mol Fe}^{2+}$ per cup for FRAP assay

Variants	TEAC (μmol Trolox per cup)	FRAP ($\mu\text{mol Fe}^{2+}$ per cup)
<i>Haberlea rhodopensis</i> L. infusion (for longer time period)	282.7 \pm 26.1	693.8 \pm 82.2
<i>Haberlea rhodopensis</i> L. infusion (for 5 min)	36.8 \pm 3.9	75.9 \pm 34.8
<i>Hibiscus sabdarrifa</i> L. infusion (for longer time period)	183.8 \pm 20.3	446.5 \pm 56.4
<i>Hibiscus sabdarrifa</i> L. infusion (for 5 min)	144.6 \pm 10.9	386.3 \pm 61.4

FRAP assay

The antioxidant efficiency evaluated by the FRAP method was expressed as $\mu\text{mol Fe}^{2+}$ per gram of tea, of each fraction (water-soluble and lipo-soluble) (Table 3). The highest and the lowest TAC were those of Green tea (North Vietnam cultivation) and Rooibos, respectively. There are no considerable differences from the TEAC assay results. However, the FRAP assay does not measure thiol antioxidants, such as glutathione. FRAP actually measures only the reducing capability based on the ferric ion, which is not relevant to antioxidant activity mechanistically and physiologically (Prior et al., 2005).

We obtained higher TAC values by the FRAP method than by the TEAC method. According to TEAC assay (Table 1), the antioxidant potential of three herbal teas (Common St John's wort, Oregano and Garden sage) was close to that of Green tea. The same was true for four herbal teas (Common St John's wort, Garden sage, *Haberlea*, and Oregano) according to the FRAP assay (Table 3).

Since teas are not consumed as dry substances, it is reasonable to evaluate whether brewing conditions could influence the final antioxidant capacity in a tea cup. We tested teas prepared from *H. rhodopensis* and Roselle tea by soaking tea bags in cups with 200 ml of boiling water each for 5 min and for ca. 30 min of infusion (commonly used domestic methods of preparation). We found a clear correlation between infusion time and the antioxidant capacity of the resulting beverage (Table 4).

Conclusions

The present study shows that a correlation between the concentration of phenolic compounds and the antioxidant capacity is not obligatory for commercial herbal teas. This could be explained by the differences in the composition and concentration of antioxidant compounds. The highest antioxidant capacities were found in commercial samples of green tea (North Vietnam cultivation), Tikanium and Green tea. According to TEAC assay, the antioxidant potential of

three herbal teas (Common St John's wort, Oregano, and Garden sage) was close to that of Green tea. The same was true for four herbal teas (Common St John's wort, Garden sage, *Haberlea*, and Oregano) according to the FRAP assay. Maximum antioxidant capacity and, hence, maximal potential health benefits could be obtained from 30 min infusions of Roselle tea and especially *H. rhodopensis* leaves. This makes cultivated *Haberlea rhodopensis* a valuable candidate as a potential ingredient for new blends of herbal tea with high antioxidant capacity.

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