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Total Phenols and Antioxidant Activities of Some Herbal Teas and *In Vitro* Bioavailability of Black Tea Polyphenols

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Abstract: In recent years, studies on phenolic compounds and antioxidant activities of foods have increased due to the inverse relationship between degenerative diseases and consumption of polyphenol rich foods. To clarify the controversy related to if antioxidant activity can be supplied *in vivo,* the necessity for data on bioavailability of phenolic compounds are considered. The aim of the study was to determine total phenols and antioxidant activities of sage, linden flower, fresh nettle, dried nettle leaves and black tea infusions which are popular beverages in the Mediterranean region and also to evaluate antioxidant activities of standard phenolic compounds (catechin, ferulic acid, quercetin) and Trolox for comparison. Moreover, another goal was to determine *in vitro* bioavailability of black tea. Both black tea infusion and black tea dialysate inhibited ABTS radical cation oxidation by 99.43% and 42% respectively. Infusions of sage, linden flower, fresh nettle, dried nettle leaves inhibited ABTS radical cation oxidation by 39.61%, 96.70%, 70.80% and 95.50% respectively. Although total phenols of black tea dialysate decreased by 96.48%, total antioxidant activity decreased by 57.76%. Ten μM catechin and 10μM quercetin showed 100% inhibition on ABTS radical cation oxidation. However, Trolox and ferulic acid showed 100% inhibition at 20 μM concentrations. Total antioxidant activity of a cup of black tea was found to be equal to 10 μM of quercetin, 10 μM of catechin, 20 μM of Trolox and 20 μM of ferulic acid. Total antioxidant activity of black tea dialysate was slightly higher than both 5 μM of Trolox and 5 μM of ferulic acid.

Key words: Black tea, sage, linden flower, fresh nettle, dried nettle leaves, black tea dialysate, total phenols, *in vitro* bioavailability, total antioxidant activity

Bazı Bitkisel Çayların Toplam Fenolik Madde İçerikleri, Antioksidan Aktiviteleri ve Siyah Çay Polifenollerinin *in vitro* Biyoyararlılığı

Özet: Son yıllarda fenolik bileşikler ve gıdaların antioksidan aktiviteleri üzerine yapılan çalışmaların sayısındaki artış, polifenollerce zengin gıdaların tüketimi ile dejeneratif hastalıklar arasında negatif ilişkinin saptanmasına bağlanmaktadır. Bu ilişkinin *in vivo* koşullarda sağlanıp sağlanamayacağını açıklığa kavuşturmak için fenolik bileşiklerin biyoyararlılığına ait çalışmalara gereksinim olduğu belirtilmektedir. Bu çalışmanın amacı Akdeniz Bölgesinde popüler olarak tüketilen adaçayı, ıhlamur, ısırgan otu, kurutulmuş ısırgan out ve siyah çayın toplam fenolik madde konsantrasyonları ile antioksidan aktivitelerini saptamak ve standart fenolik bileşikler (kateşin, ferulik asit, kuersetin) ile Troloksun antioksidan aktiviteleriyle karşılaştırmaktır. Çalışmanın bir diğer amacı ise siyah çaydaki fenolik bileşiklerin *in vitro* biyoyararlılığının belirlenmesidir. Siyah çay ve siyah çay dializatının ABTS radikal oksidasyonu üzerine inhibisyonları sırasıyla % 99.43 ve % 42 olarak saptanmıştır. Adaçayı, ıhlamur, ısırgan otu ve kurutulmuş ısırgan otu ile elde edilen çaylar ise ABTS radikal oksidasyonunu sırsıyla %39.61, % 96.70, %70.80 ve %95.50 düzeylerinde inhibe etmişlerdir. Siyah çay dializatının toplam fenolik madde içeriğindeki azalma % 96.48 olmasına rağmen toplam antioksidan aktivitedeki azalma % 57.76 olarak saptanmıştır. On μM kateşin ve 10 μM kuersetin ABTS radikal oksidasyonunu %100 inhibe etmiş, oysa Troloks ve ferulik asit için aynı inhibisyon 20 μM konsantrasyonda saptanmıştır. Bir fincan çayın toplam antioksidan aktivitesi 10μM kuersetin, 10μM kateşin, 20 μM Troloks ve 20 μM ferulik aside eşdeğer bulunmuştur. Siyah çay dializatının toplam antioksidan aktivitesi ise 5 μM Troloks ve 5 μM ferulik asitten biraz düşük olmuştur.

Anahtar kelimeler: Siyah çay, adaçayı, ıhlamur, ısırgan otu, kurutulmuş ısırgan otu, siyah çay dializatı, toplam fenoller, *in vitro* biyoyararlılık, toplam antioksidan aktivite

1. Introduction

Today new concepts in nutritional sciences are of particular importance. The concepts of food are changing from a past emphasis on survival, hunger satisfaction to an emphasis on the promising use of foods to promote better health and well-being. The most recent knowledge in biochemistry, cell biology and physiology supports the hypothesis that diet also controls and modulates various functions in the body, so participates in the maintenance of the state good health necessary to reduce the risk of some diseases.

Phenolic compounds are widely distributed in plant foods and therefore important constituents of the human diet. The term of phenolic compounds refers to the main classes of secondary metabolites in plants. Several thousand molecules have been identified in various plant species. They are extremely diverse. For example phenolic diterpenes have been identified in herbs and species (Robards et al., 1999) while the main constituents of green tea are catechins such as (-)- epigallocatechin, (-)-epicatechin, epicatechin gallate and epigallocatechin gallate plus flavonols and flavones including rutin, quercetin, kaempferol and apigenin (Karakaya and El, 1999, Ferrara et al., 2001). They are closely related with sensory and nutritional quality of foods derived from plant sources. Phenolic compounds, at low concentration, may act as an antioxidant and protect foods from oxidative deterioration. However at high concentrations, they or their oxidation products may interact with proteins, carbohydrates and minerals. Phenolic compounds are important by their contribution to human health with their multiple biological effects such as antioxidant activity, antimutagenic and/or anticarcinogenic activities, and antiinflammatory action (Grimmer et al., 1992, Xie, et al., 1993, Stavric, 1994, Shahidi and Naczk, 1995, Hollman and Katan, 1997, Parr and Bolwell, 2000).

The results of epidemiologic studies showed the inverse relationship between coronary heart diseases and flavonoid consumption (Hertog, et al., 1993, Hertog et al., 1995, Knekt et al., 1996).

In recent years, the number of studies which are conducted to determine antioxidant activity of phenolic compounds have increased due to the possible role of reactive oxygen species in the pathogenesis of degenerative diseases such as atherosclerosis, cancer and chronic inflammation (Halliwell, 1994). In this respect, antioxidant activities of phenolic compounds extracted from especially tea (Vinson, et al., 1995a, Cao, et al., 1996), fruits and vegetables (Vinson, et al., 1995b,Wang, et al., 1996, Miller and Rice-Evans, 1997, Prior et al., 1998, Karakaya et al., 2001,), red and white wines (Kanner, et al., 1994, Vinson and Hontz, 1995, Hurtado et al., 1997, Soleas, et al., 1997, Ghiselli et al., 1998, Gündüç and El, 2003) have been intensively studied using *in vitro* methods. The results of these studies showed that phenolic compounds are powerful antioxidants. However, there is still a controversy whether *in vitro* similar effects can be obtained *in vivo*, because the lack of knowledge concerning whether phenolic compounds can stay at sufficient time with efficient chemical forms in human body. To clarify this controversy, the necessity for data on absorption and metabolism of phenolic compounds in gastro-intestinal tract are considered.

The use of herbs and herbal teas as medicines has played an important role nearly every culture on earth. Tea infusions are consumed by two thirds of the world's population. Tea is for the most part of the world simply considered a tasteful drink, but the scientific community has recently re-discovered the therapeutic potential of this beverage (Wargovich et al., 2001, Luczaj and Skrzydlewska, 2005).

The aim of the study was to determine total phenols and antioxidant activities of sage, linden flower, fresh nettle, dried nettle leaves and black tea infusions which are popular beverages in the Mediterranean region and also to evaluate antioxidant activities of standard phenolic compounds (catechin, ferulic acid, quercetin) and Trolox for comparison. Moreover, another goal was to determine *in vitro* bioavailability of black tea.

2. Materials and Methods

2.1. Materials

Black tea (tea bag: 2.5 g/250 ml of water), sage (5g/200 ml of water), linden flower (5 g/200 ml of water), fresh nettle (20g/200 ml of water), dried nettle leaves (5 g/200 ml of water), Trolox (analog of synthetic vitamin E; 5, 10, 15, 20 μM), quercetin (5, 10, 15, 20 μM), ferulic acid (5, 10, 15, 20 μM) and catechin (5, 10, 15, 20 μM) were used as materials.

2.2. Chemicals

(+)-catechin hydrate (C-1251), Folin-Ciocalteu phenol reagent (F-9252), ferulic acid (F-3500), quercetin (Q-0125), pepsin (P-7000), pancreatin, bile-extract (B-8631), Trolox (23,881-3), ABTS [(2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], dialysis tubing (molecular

weight cut-off of 12,000 Da; Catalog No: 9777) were purchased from Sigma-Aldrich Co. All other chemicals were of the highest quality available.

2.3. Methods

Preparation of teas: Two hundred of milliliters of boiled water were poured on to 5 g of herbs. Afterwards, infusions were allowed to stew for five minutes. Two hundred and fifty milliliters of boiled water was used for preparation of black tea infusion.

Determination of total phenols: Total phenols were determined according to Folin-Ciocalteu method (Singleton and Rossi, 1965) by using (+)-catechin hydrate as the standard and the results were given as catechin equivalents (CE). Analyses were done in triplicate.

Determination of *in vitro* bioavailability: *In vitro* bioavailability of total phenols was determined according to Gil-Izquierdo et al. (2002), by modeling pepsin-HCl digestion in stomach and pancreatin- bile extract digestion in small intestine. For pepsin-HCl digestion, 31,500 units of pepsin were added to the samples (200 ml). The pH was adjusted to 2 by addition of concentrated HCl and then sample was incubated in a 37ºC shaking water bath for 2 h. The pepsin digest was transferred into a beaker. Segment of cellulose dialysis tubing containing 25 ml water and the amount of $NaHCO_3$ equivalent to the titratable acidity was placed in the baker. The baker was sealed with parafine film and incubated in a 37ºC shaking water bath until the pH reached about 5. Five milliliters of pancreatin (4g/l)- bile extract (25g/l) mixture was added to a beaker and incubation was continued for an additional 2 h. At the end of the incubation period the dialysis tubes were removed, rinsed with distilled water, and the dialysates analyzed. Analyses were done in triplicate.

Determination of total antioxidant activity: Total antioxidant activity of the samples was determined by using improved ABTS radical cation decolorization assay (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Afterwards, the $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30ºC. After addition of 1.0 ml of diluted $ABTS^{\cdot+}$ solution to 10 µl of samples, the absorbance reading was taken at 30ºC exactly 1 min. after initial mixing and up to 6 min. Following the absorbance readings, percent inhibition of oxidation was calculated for each sample. All analyses were performed in triplicate.

3. Results and Discussion

Total phenol contents (TP) and total antioxidant activities (TAA) of herbal tea infusions, black tea and black tea dialysate were shown in Table 1.

Table 1. Total Phenols and Total Antioxidant Activities of Herbal Tea Infusions, Black Tea and Black Tea Dialysate (n=12)

Samples	**TP (CE equivalents)**		**TAA**
	µM	**mg/l**	**(%)**
Sage	151.88 ± 11.2 [a]	44.96	39.61 ±1.60[a]
Linden Flower	108.78 ± 8.2	32.22	96.70 ± 0.50
Fresh nettle	296.75 ± 14.6	87.90	70.80 ± 0.30
Dried nettle leaves	98.21 ± 2.9	29.09	95.50 ± 1.20
Black tea	126.48 ± 26.0	37.46	99.43 ± 0.96
Black tea dialysate (dialysed fraction)	4.46 ± 2.1	1.32	42.00 ± 8.00

a: ± standard deviation

Total phenol contents of sage, linden flower, fresh nettle, dried nettle leaves and black tea were found as 44.96 mg/l, 32.22 mg/l, 87.90 mg/l, 29.09 mg/l and 37.46 mg/l respectively. Among the teas tested, infusions of fresh nettle had the highest total phenol content followed by sage, black tea, linden flower and dried nettle leaves. However, the infusions of black tea showed the highest antioxidant activity (99.43 %). Total

antioxidant activities of teas from the highest to the lowest were in the order of black tea, linden flower, dried nettle leaves, fresh nettle and sage. Total phenol content of black tea dialysate was found to be 1.32 mg/l.

Both black tea and black tea dialysate inhibited ABTS radical cation oxidation by 99.43% and 42% respectively. It was reported that major polyphenols in black tea were thearubigins (12-18%), theaflavins (3-6%), phenolic acids (10-12%), flavanols (3-10%) and flavonols (6-8%) (7,29,33). Quercetin and kaempferol contents of black tea were found as 34.8 μg/l and 110 μg/l respectively (Karakaya and El, 1999). Strong inhibition effect of black tea on ABTS radical cation (99.43%) can be explained clearly by its polyphenol content mentioned above. Furthermore, determination of 42% inhibition on ABTS radical for black tea dialysate is important. Because this finding represents that antioxidant effect can be supplied after absorption in the small intestine. Although total phenols of black tea dialysate decreased by 96.48 %, total antioxidant activity decreased by 57.76%. This difference can be due to the different antioxidant capacities of each phenolic compound in tea polyphenols or new compounds which are occurred from metabolic conversation of phenolic compounds. The relation between chemical structure of phenolics and their antioxidant activity has been of considerable interest. A single hydroxy substituent generates little or no antioxidant activity. Antioxidant activities of phenolic acids increase by the addition of hydoxyl group to the molecule. However antioxidant activity of flavonoids strongly related with the position and the degree of hydroxylation of the molecule. Although hydroxyl substitution on the A ring has no effect on antioxidant activity hydroxyl group on the B ring is responsible from the antioxidant activity. Nevertheless, the number of hydroxy substitution on the B ring has important effect on antioxidant activity. For example antioxidant activities of flavanons, naringenin and hesperidin, which have only one hydroxyl substititue can be negligible. In contrast flavonoids that contain 3'-4' dihydroxy substitution on the B ring possess strong antioxidant activity (Miller and Rice-Evans, 1997, Robards et al., 1999). Another explanation is metabolism of food polyphenols into new compounds. Metabolism of food polyphenols such as by deconjugation and reconjugation reactions plays an important role to obtain *in vivo* antioxidant effect besides their absorption in small intestine (Karakaya, 2004). Otholf et al. (2003) used 4g of black tea solids provided 4.3 mM phenols as supplements. Following consumption of black tea solids, they found hippuric acid as a metabolite of catechins, thearubigins, theaflavins and 3-hydroxyphenylacetic acid as a metabolite of quercetin-3-rutinoside. Hippuric acid has no antioxidant activity because it has no hydroxyl group. Phenylacetic acids have antioxidant activity *in vitro* that is similar to that of vitamin E, but lower than that of the parent compound quercetin.

Wang et al. (1998) reported that rosmarinic acid and luteolin -7-*O*-*β* glucopyranoside were the most active antioxidants of sage. They also stressed that rosmarinic acid was powerful antioxidant against DPPH and ABTS radicals. Contrary to, we found that infusion of sage was weak or moderate antioxidant against ABTS radical. This difference can be explained by the different extraction methods, extraction solvents (ethanol and water) and concentrations (20 g/l and 600 g/l) used in two studies. Since, widespread consumption pattern of sage was herbal tea form we used infusions of sage in our study. Furthermore Lu and Foo (2001) reported that rosmarinic acid derivatives of sage were potent antioxidants, while the flavonoids luteolin and apigenin glycosides, possessed comparatively weak to moderate activities. Antioxidant activities of the infusions of linden flower, dried nettle leaves and fresh nettle containing different amounts of total phenols, with the exception of sage, were shown in Figure 1.

There was a sharp increase (slope: 2.08) in the inhibition of ABTS radical by dried nettle leaves with the concentration range of total phenols changed between 24.55 μM and 32.74 μM (Figure 1). Above the concentration of 32.74 μM, the rate of increase in total antioxidant activity was comparatively small. Although inhibition effects of dried nettle leaves and linden flower were relatively similar, increase in total antioxidant activity of linden flower was slower than that of dried nettle leaves. It was shown that water extracts of nettle had an antihypertensive, anti-inflammatory, antirheumatic and acute diuretic

effects (Gülçin et al., 2004). Moreover, fresh nettle showed the antimutagenic effect (46.32%) against sodium azid mutagenicity in *S. typhimurium* TA 100 (Karakaya and Kavas, 1999). Gülçin et al. (2004), reported that nettle had a powerful antioxidant activity against free radical $DPPH^{\cdot}$, superoxide anion, hydrogen peroxide and showed metal chelating activity. Similarly Toldy et al. (2005) reported that nettle supplementation reduced the free radical concentration and increased the DNA binding of AP-1 in the brain of Wistar rats. Therefore researchers evaluated that nettle was an effective antioxidant and possible antiapoptatic supplement promoting cell survival in the brain. A few studies were conducted on therapeutic effects of linden flower. In the study of Mathuda et al. kaempferol moiety of Tilia argentea was found to show hepatoprotective effect in mice (Mathuda et al., 2002). Our previous study showed that kaempferol content of linden flower was 565 µg/100g (Karakaya and El, 1999). In this respect, linden flower might be regarded as powerful antioxidant and hepatoprotective agent.

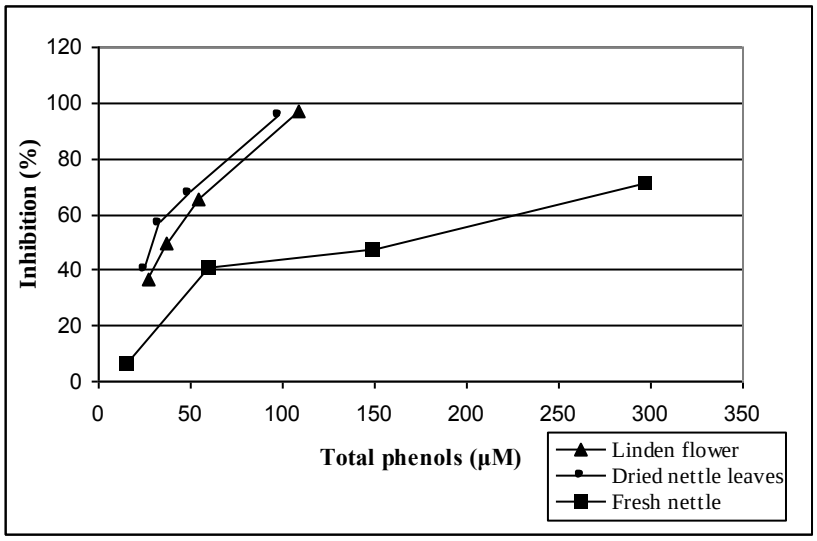

Figure1. Total antioxidant activity of linden flower, dried nettle leaves and fresh nettle containing different concentration of total phenols

Antioxidant effects of Trolox, quercetin, ferulic acid and catechin were shown in Figure 2.

Ten µM of catechin and 10µM of quercetin showed 100% inhibition on ABTS radical cation oxidation. However, Trolox and ferulic acid showed 100% inhibition at 20 µM concentrations.

Total antioxidant activity of a cup of black tea was found to be similar to 10 µM of quercetin, 10 µM of catechin, 20 µM of Trolox and 20 µM of ferulic acid. Total antioxidant activity of black tea dialysate was slightly higher than both 5 µM of Trolox and 5 µM of ferulic acid (Figure 3).

Antioxidant activity of a cup of linden flower was found to be equal to 20 µM ferulic acid and higher than that of 15 µM Trolox (Figure 4). Similarly antioxidant activity of a cup of the infusion of dried nettle leaves was higher than those of 5 µM catechin, 5 µM quercetin, 15 µM ferulic acid and 15 µM Trolox (Figure 4). However antioxidant activity of a cup of sage was slightly higher than those of 5 µM ferulic acid and 5 µM Trolox. Antioxidant activity of an infusion of a cup of fresh nettle, equal to 10 µM ferulic acid, was lower than that of dried nettle leaves.

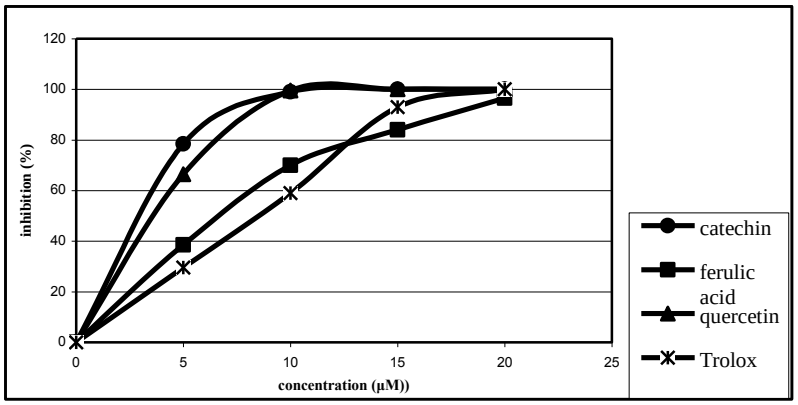

Figure2. Inhibition effects of Trolox, quercetin, ferulic acid and catechin on $ABTS^{\cdot+}$ oxidation

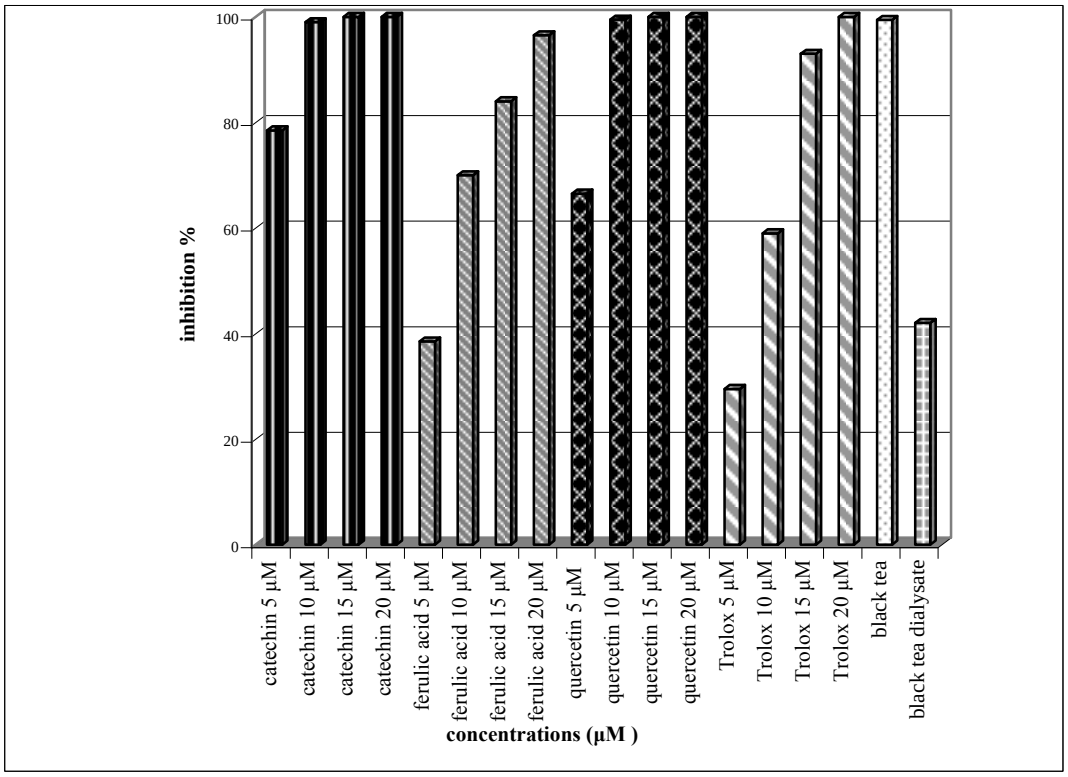

Figure 3. Comparison of total antioxidant activities of black tea, black tea dialysate and standard phenolic compounds

In conclusion we quantified that decrease in total antioxidant activity of black tea was about 58% following absorption. Total antioxidant activity of a cup of black tea was equal to 10 µM of quercetin, 10 µM of catechin, 20 µM of Trolox and 20 µM of ferulic acid. Total antioxidant activity of black tea dialysate was slightly higher than both 5 µM of Trolox and 5 µM of ferulic acid. All of the herbal teas showed antioxidant activities equal or slightly lower than those of standard phenolic compounds and Trolox. Sage had the lowest antioxidant activity. In this study, we determined only *in vitro* bioavailability of total tea polyphenols in order to evaluate the absorption of polyphenols and antioxidant activity of the dialysate. Studies on *in vitro* bioavailability of herbal tea polyphenols are in progress.

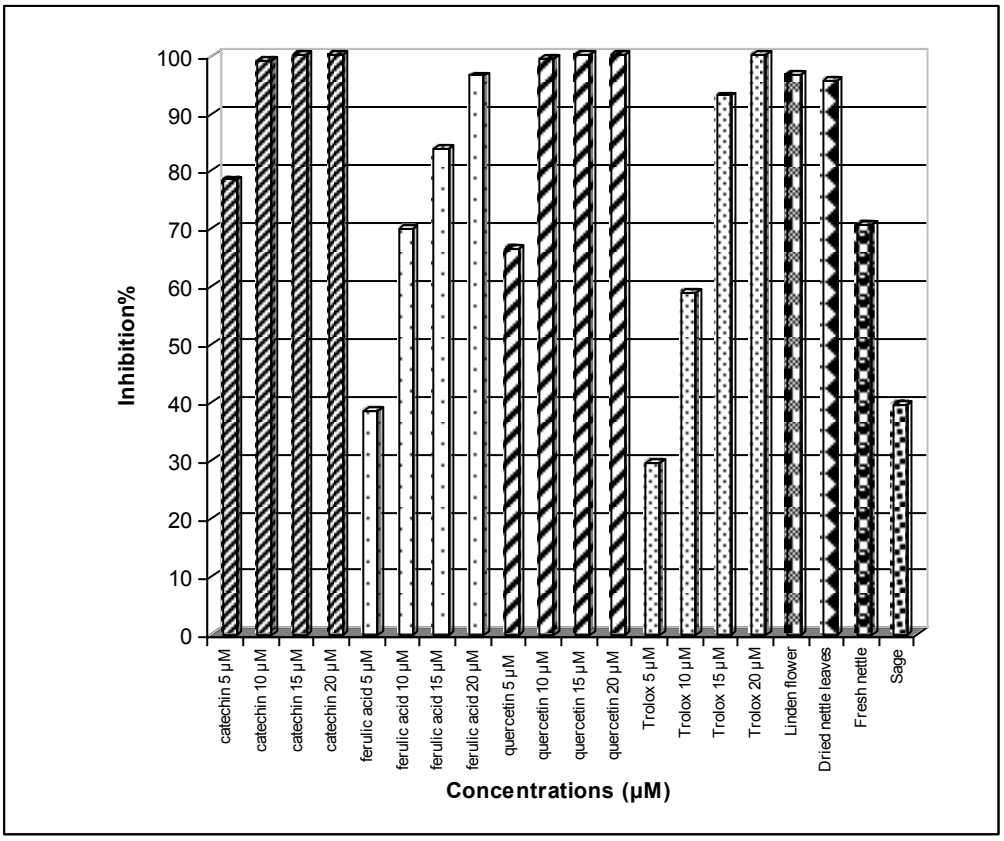

Figure 4. Comparison of total antioxidant activities of the infusions of linden flower, dried nettle leaves, fresh nettle, sage and standard phenolic compounds

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