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Food Chemistry 94 (2006) 550–557
Food Chemistry
www.elsevier.com/locate/foodchem

Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols

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Received 21 October 2004; received in revised form 3 December 2004; accepted 9 December 2004

Abstract

The total phenolic content and related total antioxidant capacity of 70 medicinal plant infusions was analyzed. Infusions were prepared in common way in which teas are prepared for human consumption. The total phenolics were measured by Folin–Ciocalteau assay. The total antioxidant capacity was estimated by Ferric Reducing/Antioxidant Power (FRAP) assay. To make practical comparison of relative antioxidant potential of phenolics extracted from selected medicinal plants, the phenol antioxidant coefficient (PAC) was calculated for each infusion. The total phenolic content of medicinal plant infusions ranges from 9 to 2218 mg/L. The FRAP range from 0.06 to 25 mM/L. There was significant linear correlation between total phenolic content and FRAP. According to their antioxidant capacity, 70 medicinal plant extracts can be divided in five groups: (a) very low FRAP (<1 mM/L) $n = 9$; (b) low FRAP (1–5 mM/L), $n = 37$; (c) good FRAP (5–10 mM/L), $n = 15$; (d) high FRAP (10–20 mM/L), $n = 8$; and (e) very high FRAP (>20 mM/L), $n = 1$ medicinal plant extract. The PAC was ranging from 1.1 to 3.9 (average 2.4). The best results were obtained for *Melissae folium* infusions: high phenolic concentration, very high FRAP (>20 mM/L) and PAC > 3. The effect of infusion time and infusion temperature on the phenolic content, FRAP, and free radical scavenging ability was tested. DPPH radical scavenging ability of *Melissae folium* phenolics was similar to (+)-catechin but not as good as for quercetin. Compared to Trolox and vitamin C, *Melissae folium* phenolics were more efficient free ABTS radical scavengers. The results indicate that *Melissae folium* infusions could be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea.

Keywords: Medicinal plants; Infusions; *Melissa officinalis* L.; FRAP; DPPH; ABTS

1. Introduction

The oxidative stress, defined as "the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage" has been suggested to be the cause of aging and various disease in humans. In modern western medicine, the balance between antioxidation and oxidation is believed to be a critical concept maintaining a healthy biological system (Ahmad, 1995; Davies, 2000; Dreosti, 1991; Finkel, 2000; Sies, 1982; Tiwari, 2001). The similar concept of balance called yin-yang has existed in traditional Chinese medicine for more than 2000 years. Ou, Huang, Hampsch-Woodili, and Flanagan (2003) and Prior and Cao (2000) have shown that the effective compositions of the yin-tonic herbs are mainly flavonoids which are phenolic compounds with strong antioxidant activity. According to them the clear trend of antioxidant activity supported the hypothesis that yin in traditional chines medicine refers to antioxidant process, whereas yang relates to oxidation process. A general recommendation to the consumer is to increase the intake of foods rich in antioxidant compounds (e.g. polyphenols, carotenoids) due to their well-known healthy effects. As a consequence these evidences accelerated the search for antioxidants

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doi:10.1016/j.foodchem.2004.12.004

principles, which led to the identification of natural resources and isolation of active antioxidant molecules. Many plants have been identified as having potential antioxidant activities and their consumption recommended (Kitts, Yuan, Wijewickreme, & Hu, 2000; Lee, Hyun, Ha, Jeong, & Kim, 2003; Lee & Shibamoto, 2000; Liu & Ng, 2000; Tiwari, 2001; Velioglu, Mazza, Gao, & Oomah, 1998; Wang & Jiao, 2000). Bioactive phenols, especially bioflavonoids, are very interesting as antioxidants because of their natural origin and the ability to act as efficient free radical scavengers (Hertog, Hollman, & Van de Putte, 1993, 1995; Langley-Evans, 2000). In last two decades the number of publications on the potential health benefits of polyphenols, has increased enormously (Ahmad, 1995; Dreosti, 1991; Friedman & Kimball, 1986; Lee et al., 2003; Modun, Musić, Katalinić, Salamunić, & Boban, 2003; Pietta, 2000; Sies, 1982; Tiwari, 2001). Tea (black and green tea) is one of the most commonly consumed beverages in the world and is rich in polyphenolic compounds collectively known as the tea flavonoids (Hertog et al., 1993; Langley-Evans, 2000; Lie & Xie, 2000). The current focus is toward natural antioxidants, especially plant polyphenolics. It is of interest to investigate the antioxidant properties of herbal infusions especially those traditionally used in folk medicine.

The aim of the present study was to examine the total phenolic content and related total antioxidant potential in 70 medicinal plant infusions prepared in common way in which teas are prepared for human consumption. Total antioxidant potential has been determined using ferric reducing ability of plasma assay (FRAP) of Benzie and Strain (1996). The efficiency of extracted phenolics was evaluated using the phenol antioxidant coefficient (PAC). The free radical scavenging ability of *Melissae folium* infusions were determined using two different stable free radicals. The effect of infusion temperatures and infusion time has been considered over a range similar to that encountered in a domestic environment.

2. Experimental

2.1. Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steineheim, Germany), Merck (Darmstadt, Germany) and Kemika (Zagreb, Croatia).

2.2. Spectrophotometric measurements

Spectrophotometric measurements were performed by UV–VIS spectrophotometer 2.2. (double-beam) Specord 200 Analytik Jena GmbH, Germany.

2.3. Plant material and protocol

Samples of 70 medicinal plants were purchased from a local pharmacy. In all experiments plant infusions were prepared according to a standard protocol. To 3 g of plant material was added 200 mL of deionised water. The initial temperature of added water was 98 °C. Infusions were left to stay at room temperature without additional heating; infusion time was 30 min. The extracts were filtered and the liquid portions were analyzed for their total phenol content and their antioxidant capacity. Each sample was prepared in four repetitions. For *Melissae folium* extracts water temperatures were varied, 20 and 98 °C, to consider the effect of infusion temperature on antioxidant potential, and infusion time varied from 1 to 30 min to determine the extent to which this variable modified the antioxidant potential of tea.

2.4. Total phenol concentration

Total phenol concentration in selected medicinal plant infusions were determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965), using (+)-catechin as the standard and expressing the results as catechin equivalents (CE). The levels of total phenols in infusions determined according to the Folin–Ciocalteu method are not absolute measurements of the amounts of phenolic materials but are in fact based on their chemical reducing capacity relative to an equivalent reducing capacity of (+)-catechin. Data presented are average of four measurements.

2.5. Determination of antioxidant capacity

2.5.1. Determination of Ferric Reducing/Antioxidant Power

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma FRAP assay of Benzie and Strain (1996) as a measure of "antioxidant power". FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} form by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100–1000 μmol/L) of $FeSO_4 \times 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay the antioxidant efficiency of the antioxidant under the test was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one-electron exchange reaction. The results were corrected for dilution and expressed in μmol Fe^{II}/L. (+)-Catechin, vitamin C, Trolox and BHT were measured within 1 h after preparation. The sample to be analysed was first adequately diluted to fit within the

linearity range. All determinations were performed in triplicate.

2.5.2. Free radical scavenging ability by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl)

The DPPH radical scavenging activity of *Melissae folium* infusions was determined using the method proposed by Von Gadow, Joubert, and Hansmann (1997). Aliquot (50 μL) of the tested sample was placed in a cuvette, and 2 mL of 6×10^{-5} M methanolic solution of DPPH radical was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined after 16 min for all samples. Methanol was used to zero spectrophotometer. The absorbance of the DPPH radical without antioxidant, i.e. the control was measured daily. Special care was taken to minimize the loss of free radical activity of the DPPH radical stock solution (Blois, 1958). Methanolic solutions of pure compounds ((+)-catechin, vitamin C and quercetin) were tested too at different concentrations (× mol of antioxidant/1 mol DPPH radical).

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994)

$$\% \text{ inhibition} = [(A_{C(0)} - A_{A(t)})/A_{C(0)}] \times 100,$$

where $A_{C(0)}$ is the absorbance of the control at $t = 0$ min; and $A_{A(t)}$ is the absorbance of the antioxidant at $t = 16$ min.

2.5.3. Free radical scavenging ability by the use of a stable ABTS radical cation (2,2′azinobis-(3-ethylbenzthiazoline -6-sulphonic acid))

The free radical scavenging activity of *Melissae folium* infusions was determined by ABTS radical cation decolorization assay (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS*+) 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. Because ABTS and potassium persulfate react stoichiometrically at a ratio of 1:0.5, this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature. For the study of infusion samples the ABTS*+ solution was diluted with ethanol to an absorbance of 0.70(±0.02) at 734 nm and equilibrated at 30 °C. Reagent blank reading was taken (A_0). After addition of 2.0 mL of diluted ABTS*+ solution ($A_{734\ nm} = 0.700 \pm 0.020$) to 20 μL of *Melissae folium* phenolic or antioxidant compounds (final concentration 0–15 μM) the absorbance reading was taken at 30 °C exactly 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm was calculated using above formula and decrease of the absorbance between A_0 and A_t.

2.6. Statistical analysis

The direction and magnitude of correlation between variables was done using analysis of variance (ANOVA) and quantified by the correlation factor "*r*". The *P*-values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Total phenol content of 70 medicinal plant infusions

There was a wide rage of phenol concentrations in the medicinal plant infusions analyzed as shown in Table 1. The values varied from 9 to 2218 mg CE/L of infusion (average 470 mg/L) as measured by the Folin–Ciocalteau method. It is well known that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations (Harborne, 1993). Between 70 selected medicinal plants infusions the lowest phenolics concentrations (<100 mg/L) had *Althaeae radix* < *Cetrariae lichen* ≈ *Foeniculi fructus* < *Pheseoli pericarpum* < *Visci albi herba* infusions. Only four infusions had phenolic concentrations > 1000 mg CE/L: *Melissae folium* > *Spirae herba* > *Uvae ursi folium* > *Rubi fructose folium* infusions. The highest phenol content (>2000 mg CE/L) was found in *Melissae folium* infusions. According to the Singleton and Rossi (1965) various phenolic compounds have different responses in this assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate, but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented ortho or para (Frankel, Waterhouse, & Teissedre, 1995). Since these structural features of phenolic compounds are reportedly also responsible for antioxidant activity, measurements of phenols in infusions may be related to their antioxidant properties.

3.2. Total antioxidant capacity of 70 medicinal plant infusions

As shown in Table 1, there were big differences in total antioxidant capacity (FRAP) between the selected medicinal plant infusions. The FRAP values varied from 59 to 25,234 (mean 4654) μmol Fe^{II}/L of infusion. The significant linear correlation (coefficient "*r*" = 0.9825,

Table 1
The total phenolic content and related total antioxidant capacity determined as FRAP of 70 medicinal plant, infusates

Plant material (Medicinal name)	Total phenolics (mg CE/L)[a]	FRAP[b] (μmol/L)	PAC[c]
1. *Melissae folium*	2218	25234	3.3
2. *Spiraea herba*	1136	15256	3.9
3. *Uvae ursi folium*	1122	13207	3.4
4. *Rubi fructose folium*	1050	12211	3.4
5. *Salicis cortex*	939	10892	3.4
6. *Teucrii herba*	926	8810	2.8
7. *Gerani robertiani herba*	915	10696	3.4
8. *Thymi herba*	876	9069	3.0
9. *Rubi idaei folium*	848	10025	3.4
10. *Serpylli herba*	846	10868	3.7
11. *Fragariae herba folium*	841	11022	3.8
12. *Epilobii herba*	841	7899	2.7
13. *Myrtili fructus*	743	7539	3.0
14. *Mentha piperita folium*	706	8987	3.7
15. *Veronicae herba*	686	6514	2.8
16. *Lavandulae flos*	655	7377	3.3
17. *Salviae off folium*	638	7603	3.5
18. *Luglandis folium*	631	7432	3.4
19. *Fraxini folium*	626	7129	3.3
20. *Solidaginis herba*	578	4256	2.1
21. *Farfarae folium*	561	5350	2.8
22. *Basilici herba*	561	5314	2.8
23. *Hyperici herba*	557	5127	2.7
24. *Majoranae folium*	538	4453	2.4
25. *Hederae folium*	531	5100	2.8
26. *Teraxaci folium*	502	4600	2.7
27. *Sanbuci flos*	498	4055	2.4
28. *Teucrii Montana herba*	479	4259	2.6
29. *Oleae folium*	469	3945	2.4
30. *Betulae folium*	453	3896	2.5
31. *Tiliae off. Flos*	453	3807	2.4
32. *Satureiae herba*	446	5339	3.5
33. *Chellidonii herba*	415	3401	2.4
34. *Ehinaceae herba*	409	4033	2.9
35. *Crataegi flos*	392	3025	2,2
36. *Urticae folium*	390	3168	2.4
37. *Euphrasiae herba*	370	3107	2.4
38. *Bardanae folium*	337	2337	2.0
39. *Millefolii herba*	330	3228	2.8
40. *Plantago major folium*	329	2733	2.4
41. *Verbenae herba*	325	2089	1.9
42. *Asperulae herba*	322	2557	2.3
43. *Primulae radix*	320	2197	2.0
44. *Galii veri*	314	2608	2,4
45. *Strobuli lupuli*	311	2204	2.1
46. *Morus nigra folium*	308	2360	2.2
47. *Senae folium*	298	1078	1.1
48. *Petroselini folium*	284	1318	1.4
49. *Hibisci flos*	282	3157	3.3
50. *Chamomillae flos*	279	2856	3.0
51. *Absinthii herba*	258	2228	2.5
52. *Equiseti herba*	258	2222	2.5
53. *Plantago lanc folium*	247	1727	2.0
54. *Calendulae flos*	221	1347	1.8
55. *Marubii herba*	206	1653	2.3
56. *Cantaurii herba*	191	1347	2.1
57. *Cichorii herba*	188	1408	2.2
58. *Polygoni herba*	186	1210	1.9
59. *Violae tricoloris herba*	181	846	1.4
60. *Lauri folium*	170	1260	2.2
61. *Stigmata maydis*	164	1009	1.8
62. *Verbasi flos*	150	603	1.2
63. *Malvae herba*	143	927	1.9
64. *Rosmarini folium*	136	1277	2.7
65. *Bursae pastoris herba*	128	654	1.5
66. *Visci albi herba*	97	727	2.2
67. *Phaseoli pericarpum*	58	319	1.6
68. *Foeniculi fructus*	29	142	1.5
69. *Cetrariae lichen*	29	125	1.3
70. *Althaeae radix*	9	59	1.9

Medicinal plants were purchased from local pharmacy. Aqueous extracts were prepared as infusions.
(3 g of dried plant material was infused with 200 mL of boiled water). Infusion time was 30 minutes.

[a] mg CE/L – miligram catechin equivalent per liter of infusate.
[b] FRAP – ferric reducing/antioxidant power.
[c] PAC- Phenol antioxidant coefficient, calculated as ratio FRAP (μM/L) / total phenolics (μM CE/L).

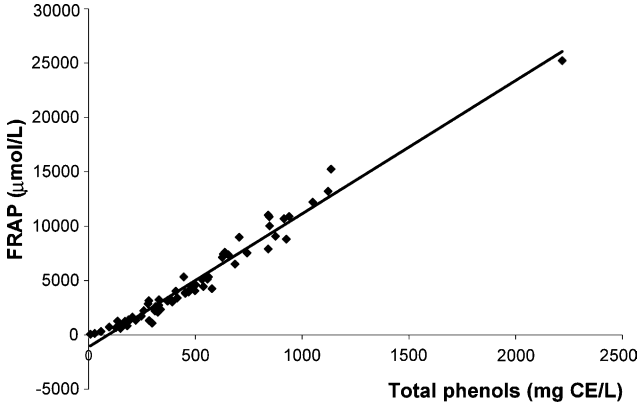

Fig. 1. Linear correlation between the amount of total phenols and antioxidant capacity (FRAP). Correlation coefficient "*r*" = 0.9825: 95% confidence interval: 0.9719–0.9891.Coefficient of determination (r^2) = 0.9653. The two-tailed *P* value is <0.0001, considered extremely significant.

and two-tailed "*P*"-value <0.0001) was confirmed between total phenolics and related FRAP of medicinal plant extracts (Fig. 1). Ou et al. (2003) have not found linear response between total phenolics and antioxidant activity, but authors used oxygen radical absorbance capacity (ORAC) assay. There are many methods to determine antioxidant capacity. These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods particular antioxidants have varying contributions to total antioxidant potential (Cao & Prior, 1998). In this study we used FRAP assay because it is quick and simple to perform, and reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present (Benzie, Wai, & Strain, 1999). This method was initially developed to assay plasma antioxidant capacity, but can be used to measure the antioxidant capacity from a wide

range of biological samples and pure compounds to fruits, wines, and animal tissues (Ghiselli, Nardini, Baldi, & Scaccini, 1998; Katalinić, Miloš, Modun, Musić, & Boban, 2004; Modun et al., 2003; Tsai, McIntosh, Pearce, Camden, & Jordan, 2002). According to their reducing ability/antioxidant power, 70 medicinal plant infusions can be divided in five groups: (a) very low FRAP (<1 mM/L), $n = 9$; (b) low FRAP (1–5 mM/L), $n = 37$; (c) good FRAP (5–10 mM/L), $n = 15$; (d) high FRAP (10–20 mM/L), $n = 8$; and (e) very high FRAP (>20 mM/L) $n = 1$. In sharp contrast, when compared to medicinal plants with high or very high FRAP, the positive properties of the medicinal plants with very low FRAP are very unlikely related to their antioxidant capacity. The antioxidant coefficient (PAC) of selected medicinal plants, calculated as ratio between FRAP (μM/L) and total phenolics (μM CE/L), was used for quick comparison of antioxidant efficiency of total phenolics from different plant infusions. The calculated PAC differed from plant to plant ranging from 1.1 to 3.9 (average 2.4). Among 16 medicinal plant infusions with PAC > 3, was one infusion from the group with low FRAP, six from the group with good FRAP and all medicinal plants infusions with high and very high FRAP. Although extracted phenolics in *Hibisci flos* infusions had very good antioxidant properties (PAC > 3) this plant infusion cannot be considered as rich source of natural antioxidants because of low phenolic concentration and low FRAP. Medicinal plant infusions with considerable antioxidant potential and high PAC can be considered as a rich dietary source of potent antioxidants. The strongest antioxidant properties when measured with the FRAP assay had *Melissae folium* > *Spirae herba* > *Uvae ursi folium* > *Rubi fructose folium* > *Fragariae herba foliu* > *Salicis cortex* > *Serpylii herba* > *Gerani robertiani herba* and *Rubi idaei folium*. The best results were obtained for *Melissae folium* infusions: high phenolic content, very high FRAP (>20 mM/L) and PAC > 3. For that reason *Mellisa officinalis* L. can be considered the antioxidant queen between 70 selected medicinal plants.

3.3. Effect of infusion time and infusion temperature on antioxidant capacity

Fig. 2. shows the effect of infusion time and infusion temperature on phenolic concetration and FRAP in *Melissae folium* infusions. Antioxidant potentials were followed over a range of infusion times from 1 to 30 min. The release of antioxidants from plant material was rapid. In first minute more than 40% of water soluble phenolics was extracted with hot (98 °C) water. The quick increase in total phenolics concentration, observed during first 5 min, was followed with adequate quick increase of FRAP in infusions prepared with hot water. Total phenolic content for *Melissae folium* infusion increased from 180 to 358 mg/g dry matter as the time increased from 1 to 5 min. About 10 min of infusion time was enough for the extraction of almost all (94%) water-soluble phenolics under the test conditions. FRAP in *Melissae folium* infusions increased in linear manner with phenolics. Preparation of *Melissae folium* infusions with hot (98 °C) and cold (20 °C) water revealed that although antioxidants were liberated from leaves into the water at both of the temperatures studied, infusions prepared at higher temperature had more than 2-fold higher antioxidant capacity determined as FRAP.

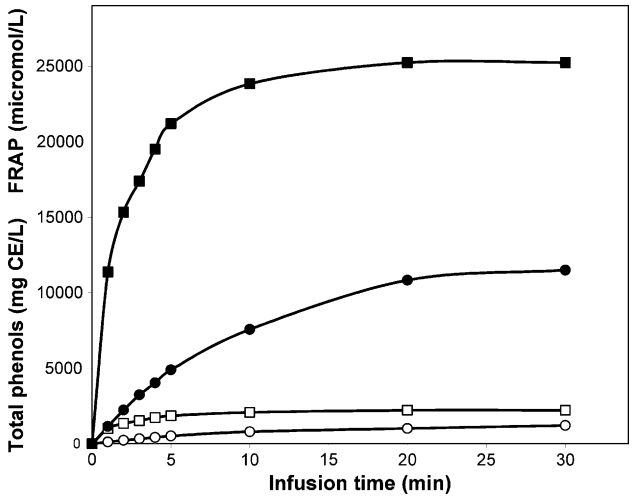

Fig. 2. The increase of total phenols and related total antioxidant capacity determined as FRAP in the *Melissae folium* infusates prepared with hot water at 98 °C (■FRAP; □ Total phenols), and infusates prepared with cold water at 20 °C (● FRAP; ○ Total phenols).

The effect of infusion temperature on free radical scavenging ability was determined with DPPH and ABTS assays. Both type of *Melissae folium* infusions had significant DPPH scavenging ability. The percentage inhibition of DPPH radical for infusions diluted with water (1:10, v/v) were 47.91% and 85.55%, respectively given for infusion temperatures 20 and 98 °C, at infusion time 30 min. The percentage inhibition of ABTS radical for infusions diluted with water (1:5, v/v) was 40.42% and 83.62%, respectively given for infusion temperatures 20 and 98 °C, and infusion time 10 min. These results also indicate that infusion temperature increase the concentration of phenolics and consequently affect the free radical scavenging ability.

3.4. Antioxidant potential of Melissae folium phenolics in comparison with other antioxidants

3.4.1. Comparison of FRAP of Melissae folium phenolics with FRAP of other antioxidants

FRAP of adequately diluted *Melissae folium* infusions was compared with FRAP of several pure antioxidants: vitamin C, Trolox, (+)-catechin and BHT (Fig. 3). The relative antioxidant efficiency of *Melissae*

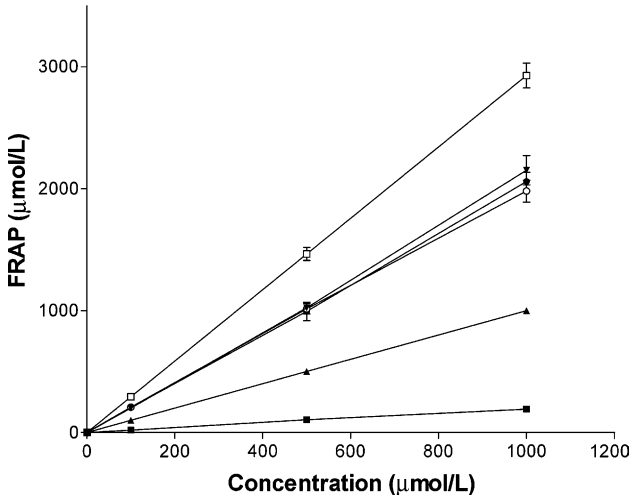

Fig. 3. Dose response line of: (□) *Melissae folium* phenolics, (○)Catechin, (●)Trolox, (▼)Vitamin C, (■)BHT, and (▲) Fe^{II} over the concentration range of 50–1000 μM, in the ferric reducing/antioxidant power test (FRAP) assay for reducing (antioxidant) activity. Each point represents the mean of three readings.

folium phenolics was 3.0, that is, on 1 mol per mol basis *Melissae folium* infusion phenolics gave thrice the signal induced by Fe^{II}, Fe^{II} representing a one electron exchange in the FRAP assay. The relative antioxidant efficiency of (+)-catechin, vitamin C and Trolox was 2.0 (Benzie et al., 1999; Katalinić et al., 2004). Compared to analysed well known natural and synthetic antioxidants, total antioxidant capacity determined as reducing power of extracted *Melissae folium* phenolics is very promising.

3.4.2. Free radical scavenging ability of Melissae folium phenolics in comparison with other antioxidants

For evaluation of free radical scavenging properties of *Melissae folium* phenolics we have used two assays: DPPH radical and ABTS radical cation assays. The results of investigation are shown in Figs. 4 and 5.

Relatively stable organic radical DPPH has been widely used in the determination of antioxidant activity of single compounds as well as the different plant extracts (Brand-Williams, Cuvelier, & Berset, 1995; Kulišić, Radonić, Katalinić, & Miloš, 2004; Yen & Duh, 1994). The addition of *Melissae folium* infusions (50 μL/2 mL) to the DPPH solution induced a rapid decrease in the optical density at 517 nm. Fig. 4 shows the effect of different concentrations of *Melissae folium* phenolics (infusion prepared at 98 °C was adequately diluted with water) in comparison with (+)-catechin, quercetin, and vitamin C on the inhibition of DPPH radical. Our investigation shows that free radical scavenging ability of *Melissae folium* phenolics was similar to (+)-catechin but not as good as for quercetin. Compared to vitamin C, *Melissae folium* phenolics were better DPPH radical scavengers, under the test conditions.

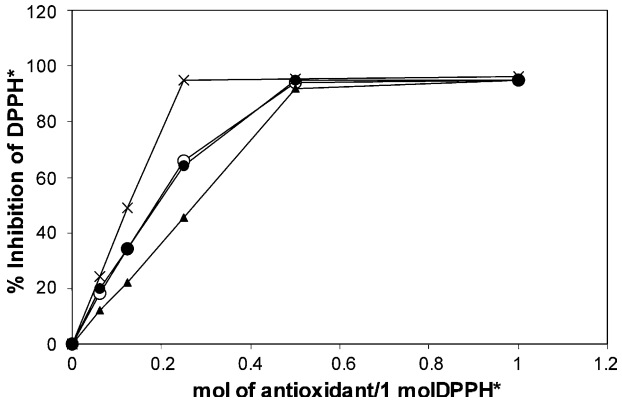

Fig. 4. The percentage inhibition of free DPPH radical in the presence of different concentration of antioxidants: (○) *Melissae folium* phenolics, (●) (+)-catechin, (▲) Vitamin C, and (×) quercetin. The concentration of antioxidant is expressed as mol of antioxidant/mol of DPPH in reaction mixture.

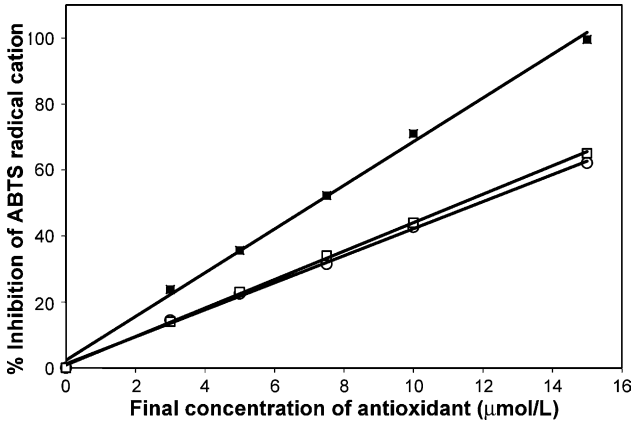

Fig. 5. Concentration–response curve for the absorbance at 734 nm for ABTS radical cation as function of concentrations of (■) *Melissae folium* phenolics, (○) Trolox and (□) Vitamin C solutions. The tested samples were diluted with water to get adequate concentrations (final concentrations were 0–15 μM). After addition of 2.0 ml ABTS radical cation solution ($A_{734\ nm}$ = 0.700 ± 0.020) to 20 μL of tested samples the absorbance reading was taken at 30 °C exactly 6 min after initial mixing.

The 50% inhibition of DPPH radical was obtained with significantly lower concentrations of *Melissae folium* phenolics compared to vitamin C. The average molar ratio between concentrations of *Melissae folium* phenolics and vitamin C, giving the same percentage of inhibition of DPPH radical in linear part of dose–response curve, was 0.6:1 under the test conditions.

The free radical scavenging ability of *Melissae folium* phenolics was determined using ABTS radical cation, too. ABTS radical cation has been often used in the evaluation of antioxidant activity of single compounds and complex mixtures of various origins (body fluids, foods, beverages, plant extracts). In this assay ABTS radical cation was generated directly in stable form using potassium persulfate. Generation of radical before

the antioxidants are added prevents interference of compounds, which affect radical formation. This modification makes the assay less susceptible to artifacts and prevents overestimation of antioxidant capacity (Sanchez-Moreno, 2002). When stable absorbance is obtained the antioxidant sample is added to the reaction medium, and the antioxidant activity is measured in terms of decolorization. The rapid release of antioxidants from plant material in infusions prepared with hot water (98 °C) resulted with strong and quick inhibition of ABTS radical cation. The dose–response curve obtained by analysis of a range of concentrations of *Melissae folium* phenolics, Trolox and vitamin C, was plotted as the percentage inhibition of the absorbance of the ABTS$*^{+}$ solution as a function of concentration of antioxidant (Fig. 5). The results indicate that *Melissae folium* phenolics were better ABTS radical cation scavengers than vitamin C or Trolox. The average molar ratio between concentrations of *Melissae folium* phenolics and vitamin C giving the same percentage of inhibition of ABTS radical cation in linear part of dose–response curve was similar as for DPPH assay. The calculated Trolox equivalent (TE) for *Melissae folium* infusions was 1200 μmol TE/100 mL of infusion. Compared with the results of Miller, Rigelhof, Marquart, Prakash, and Kanter (2000) the results indicate that one cup of *Melissae folium* infusion can contribute to dietary intake of antioxidants.

3.4.2.1. Comparison of DPPH radical and ABTS radical cation scavenging properties. Because of their high reactivity, most free radicals react rapidly with oxidizable substrates. Methods used for evaluation of radical-trapping properties often utilize stable model free radicals as indicators for radical-scavenging abilities, among which 1,1-diphenyl-2-picrilhydrazyl radical (DPPH*) and 2,2′azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation (ABTS$*^{+}$), have gained the highest popularity.

From the methodological point of view the DPPH* method is recommended as easy and accurate with regard to measuring the antioxidant activity of fruit and vegetable juices or extracts. The results are highly reproducible and comparable to other free radical scavenging methods such as ABTS (Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000). Reaction kinetics between phenols and ABTS$*^{+}$ have been found to differ from that between phenols and DPPH* over a similar range of concentrations. Campos and Lissi (1996) have suggested that this differences can be partly result of different equilibrium displacements in reaction (1) as a result of the fact that the reactions of DPPH* are carried out in the absence of added DPPH-H (i.e. the reduced form), but the reduced form ABTS is always present in the systems containing ABTS$*^{+}$ (Koleva, Niederlander, & Van Beek, 2001).

$$\text{Radical} + \text{PhOH} \leftrightarrow \text{Reduced form} + \text{PhO}^{*}(\text{Ph} - \text{phenol}) \quad (1)$$

Reactions of phenols with ABTS radical cation are usually rapid, but the reactions with DPPH radical differ from compound to compound. Three types of kinetics, rapid, intermediate and slow, have been distinguished for the reactions between DPPH radicals and phenols (Campos & Lissi, 1996; Sanchez-Moreno, 2002). We have observed rapid and strong inhibition of both, DPPH radical or ABTS radical cation, after the addition of *Melissae folium* phenolics. Aqueous extracts of *Melissae folium* dose-dependently increased DPPH free radical and ABTS free radical cation scavenging activity. Compared to vitamin C and Trolox, *Melissae folium* phenolics were stronger free radical scavengers. To evaluate contribution of each single component, knowledge of its molecular structure is required. Unfortunately, in the search of novel active compounds in complex matrixes, this is not always possible. Furthermore, the modern concept to isolate pure compounds may not achieve the desired results, as observed in natural version. Once an active principle is isolated from the natural product without its synergic colleagues to support and/or balance its action, it may lose its character as present in its natural form (Tiwari, 2001).

4. Conclusion

In conclusion, we might say that our results further support the view that some medicinal plants are promising sources of natural antioxidants. Total phenol content and total antioxidant capacity differs significantly among 70 selected medicinal plants infusions. There was significant linear correlation between phenolics concentration and FRAP in infusions. The strongest antioxidant properties when measured with the FRAP assay had *Melissae folium*, *Spirae herba*, *Uvae ursi folium*, *Rubi fructose folium*, *Fragariae herba folium*, *Salicis cortex*, *Serpylii herba*, *Gerani robertiani herba* and *Rubi idaei folium* infusions. The best results were obtained for *Melissae folium* infusions: high phenolic concentration, very high FRAP (>20 mM/L) and PAC > 3. This study showed that *Melissae folium* infusions possess a significant reducing power and free radical scavenging ability in vitro comparable with red wine or beverages like tea. The antioxidative properties in vivo of *Melissae folium* phenolics should be the objective of future research.

Acknowledgement

This work was partially supported by the Ministry of Science and Technology of the Republic of Croatia, Project 0011-003.

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