

Total antioxidant and radical scavenging capacities for different medicinal herbs

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Abstract

In this study, a total of eleven medicinal herbs (*Rhamnus frangula*, *Echinaceae herba*, *Phoeniculus*, *Malva silvestris*, *Crataegus monogyna*, *Taraxacum officinale*, *Plantago major*, *Artemisia absinthium*, *Epilobium montanum*, *Chelidonium majus*, *Melissae Folium*) from the local market were analyzed for total antioxidant and scavenging capacities. Total antioxidant capacity was analyzed using FRAP method (Benzie & Strain, 1996) and total scavenging capacity by DPPH method (Burits & Bucar, 2000; Cuendet et al., 1997). The results obtained for total antioxidant capacities varied between 23.25 – 265.5 mM Fe²⁺/L for FRAP method and for radical scavenging capacity between 2.87 – 140.19 % for DPPH method. The highest TAC_{FRAP} and TAC_{DPPH} values were obtained for *Rhamnus frangula* (5,19 mM/L) and *Echinaceae herba* (23,43%). The lower TAC_{FRAP} and TAC_{DPPH} values were identified for tomatoes *Malva silvestris* extract (0,75 mM/L and 7,32%). The good correlation between the two methods of the characterization of antioxidant capacity (FRAP and DPPH) suggest that the antioxidant compounds from analyzed medicinal herbs present both reducing power and radical scavenging capacity.

Keywords: antioxidant capacities, FRAP method, DPPH method, medicinal herbs

Introduction

Different studies have shown that free radicals present in human organism are responsible for oxidative damage to different molecules (lipids, proteins, nucleic acids) and thus are involved in the initiation phase of some degenerative illnesses. The antioxidant compounds are capable of neutralizing free radicals and may play a major role in the prevention of certain diseases such as cancer, cataracts, cerebral pathologies and rheumatoid arthritis (Clayton, 2000^[1], Madsen and Bertelsen, 1995^[2]).

The botanical plant extracts are based on the following effects: antimicrobial and antifungal effects; anti-oxidative activity, control the auto-oxidative stress caused by free radicals from blood; ameliorate the liver activity and increase the resistance at toxins; stimulate the proper enzymatic equipment activity and increase the nitrogen absorption; control the pollution by reducing the unpleasant smells and binding ammonium nitrogen.

At the cellular level, oxidation of fatty acids (FA), also referred to lipoperoxidation, is a major consequence of oxidative stress and a self-propagating biological reaction initiated by ROS which remove protons from FA (Niki et al., 2005^[3]). Lipoperoxidation severely alters mammalian cell structure and functions, and may produce toxic metabolites (Esterbauer, 1993^[4]) unless ROS are rapidly neutralized by antioxidants. At the organism level, lipoperoxidation has been implicated in deterioration of physiological functions that include growth and reproduction, as well as immunity leading to a higher susceptibility to infectious diseases (Miller and Brzezinska-Slebozinska, 1993^[5]).

Use of dietary antioxidants is recommended to limit lipoperoxidation and preserve animal health and product quality (Wood and Enser, 1997^[6]). Vitamin E is a synthetic antioxidant commonly used in animal nutrition, but its bioefficiency is limited when n - 3 PUFA intake is increased (Allard et al., 1997^[7]).

Recent investigations in the field of antioxidants have focused on naturally occurring molecules to satisfy consumer concerns over safety and toxicity of food additives. Among natural antioxidants, polyphenols are interesting since they are widely distributed in plants and exhibit various antioxidant properties (Salah et al., 1995^[8]; Bravo, 1998^[9]; Brown et al., 1998^[10]).

The antioxidant activity of plant extracts is of particular interest both because of their beneficial physiological activity on human cells and the potential they have to replace synthetic antioxidants used in foodstuffs (Amarowicz et al., 1999^[11]).

In addition to their activity as antioxidants these compounds often display biological activity of various kinds against bacteria (Barnabas and Nagarajan, 1988^[12]; Rauha et al., 2000^[13]).

In this study two methods were used to test the antioxidant activity of medicinal herbs, including one based on the evaluation of the free-radical scavenging capacity of the medicinal herbs and one based on measuring their iron-reducing capacity.

Materials and methods

Reagents and equipment

All chemicals and reagents were analytical grade or purest quality purchased from Sigma, Merck, Aldrich and Fluka. Deionized water was used. Absorption determination for FRAP and DPPH methods was made using SPECORD 205 spectrophotometer by Analytik Jena.

Samples preparation

In the present study, a total of eleven medicinal herbs from local markets were analyzed for total antioxidant capacity. The medicinal herbs samples used for determination were: *Rhamnus frangula*, *Echinaceae herba*, *Phoeniculus*, *Malva silvestris*, *Crataegus monogyna*, *Taraxacum officinale*, *Plantago major*, *Artemisia absinthium*, *Epilobium montanum*, *Chelidonium majus*, *Melissae Foliums*. For antioxidant compounds extraction were prepared ethanolic (50%) extracts in ratio 10/20. After 30 minutes all the extracts were filtered and diluted 1/10 with deionized water.

Evaluation of total antioxidant capacity (TAC) by FRAP method

FRAP method depend upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH. This ferrous tripyridyltriazine complex has an intensive blue color and can be monitored at 593 nm (Benzie and Strain, 1996^[14]).

Reagents: acetate buffer, 300mM/L, pH 3.6 (3.1g sodium acetate 3H₂O and 16 mL conc.; Acetic acid per 1L of buffer solution); 10 mM/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM/L HCl; 20 mM/L FeCl₃6H₂O in distilled water. FRAP working solution: 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL FeCl₃ solution. The working solution must

be always freshly prepared. Aqueous solution of known Fe (II) concentration was used for calibration, in a range of 0.1-0.8 mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.1, 0.2, 0.4, 0.6, 0.8 μ M/mL aqueous Fe(II) as Mohr salts solution (1mM) were mixed with 2.5 mL FRAP working solution; FRAP reagent was used as blank. The absorption was read after 10 min. at 25°C and 593 nm. All determinations were repeated for three times. Total antioxidant capacity in Fe (II) equivalents was calculated. Correlation coefficient (r^2) for calibration curve was 0.9994.

Evaluation of total antioxidant capacity (TAC) by DPPH method

Hydrogen atom – or electron-donation ability of the corresponding medicinal herbs was measured from the bleaching of the purple-colored ethanol solution of DPPH. This spectrophotometric assay uses stable 2,2'-diphenylpicrylhydrazyl (DPPH) radical as reagent. 0.5 mL of various ethanol juices extracts diluted 1/10 were added to 2.5 mL of a 1 mM ethanol solution of DPPH. After 40 min. incubation at room temperature the absorbance was read against a blank at 517 nm. TAC as inhibition of DPPH free radical in percent was calculated in following way (Burits and Bucar, 2000^[15]; Cuendet et al, 1997^[16]):

$$\text{TAC}_{\text{DPPH}} (\%) = (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}} \times 100$$

Results and Discussion

The results for total antioxidant capacity (TAC) by FRAP and DPPH methods for medicinal herbs are presented in Table 1 and more suggestive in Figures 1 and 2.

Table 1. Total antioxidant capacity (TAC) by FRAP and DPPH methods for medicinal herbs

Nr. crt.	Samples	TAC-FRAP, mM/L	TAC-DPPH, %
1	<i>Rhamnus frangula</i>	5,19	16,24
2	<i>Echinaceae herba</i>	3,36	23,43
3	<i>Phoeniculus</i>	4,55	21,13
4	<i>Malva silvestris</i>	0,75	7,32
5	<i>Crataegus monogyna</i>	3,58	12,02
6	<i>Taraxacum officinale</i>	3,24	21,87
7	<i>Plantago major</i>	4,16	17,17
8	<i>Artemisia absinthium</i>	3,50	22,93
9	<i>Epilobium montanum</i>	4,28	21,87
10	<i>Chelidonium majus</i>	1,84	11,17
11	<i>Melissae Folium</i>	2,99	14,71

Between all medicinal herbs the highest TAC_{FRAP} value is obtained for *Rhamnus frangula* (5,19 mM/L), followed by *Phoeniculus* (4,55 mM/L), *Epilobium montanum* (4,28 mM/L) and *Plantago major* (4,16 mM/L). The medium values were registered for: *Crataegus monogyna* (3,58 mM/L), *Artemisia absinthium* (3,50 mM/L), *Echinaceae herba* (3,36 mM/L), *Taraxacum officinale* (3,24 mM/L) and *Melissae Folium* (2,99 mM/L). The smallest value was noticed for *Malva silvestris* (0,75 mM/L).

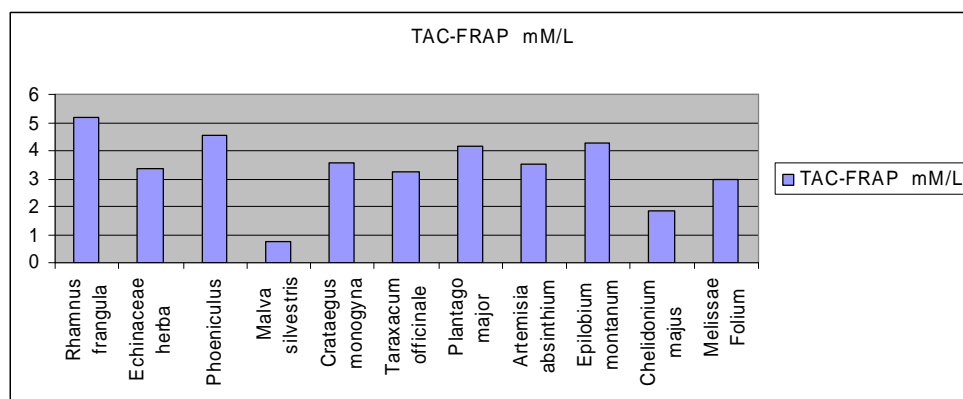


Figure 1. Total antioxidant capacity (TAC) by FRAP method for medicinal herbs

Radical scavenging capacity determined by DPPH methods (read after 40 min.) for analyzed samples are presented in Table 1 and more suggestive in Figure 2.

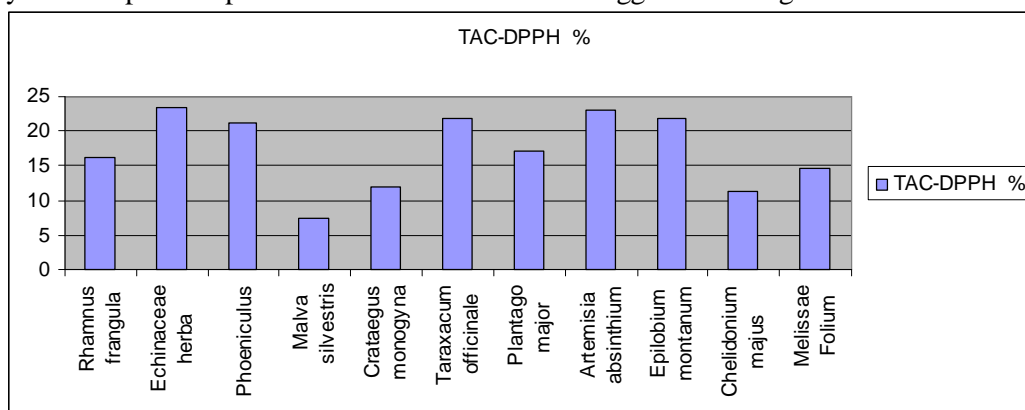


Figure 2. Total antioxidant capacity (TAC) by DPPH method for medicinal herbs

The highest TAC radical scavenging capacity values (DPPH) was identified for Echinaceae herba (23,43%), followed by Artemisia absinthium (22,93%), Epilobium montanum (21,87%), Taraxacum officinale (21,87%) and Phoeniculus (21,13%). The medium values were identified for: Plantago major (17,17%), Rhamnus frangula (16,24%), Melissae Folium (14,71%). Chelidonium majus (11,17%) and Malva silvestris (7,32%) present the lower values.

TAC_{FRAP} is a measure of the presence in medicinal herbs of the compounds with reducing power and TAC_{DPPH} is a measure of the presence in the medicinal herbs of the compounds with radical scavenging capacity. Some of these compounds can to present both of these properties. For the studied medicinal herbs the values obtained for TAC_{FRAP} are in good correlation with those for TAC_{DPPH} , with a correlation coefficient $r^2 = 0.7705$ (Figure 3). This good correlation coefficient suggests that the antioxidant compounds for analyzed medicinal herbs in our study present both reducing power and radical scavenging capacity.

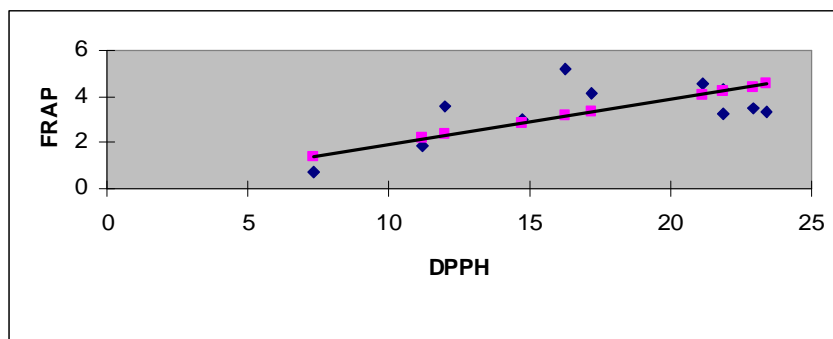


Figure 3. Correlation between TAC_{DPPH} and TAC_{FRAP} for medicinal herbs

Conclusions

The highest TAC_{FRAP} and TAC_{DPPH} values were obtained for *Rhamnus frangula* (5,19 mM/L) and *Echinaceae herba* (23,43%).

The lower TAC_{FRAP} and TAC_{DPPH} values were identified for tomatoes Malva silvestris extract (0,75 mM/L and respectively 7,32%).

The good correlation between the two methods of the characterization of antioxidant capacity (FRAP and DPPH) suggest that the antioxidant compounds from analyzed medicinal herbs present both reducing power and radical scavenging capacity.

Acknowledgements

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