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Assessing the influence of various factors on antioxidant activity of medicinal herbs

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Abstract

The purposes of this study were: (1) to assess the antioxidant activity of fifteen medicinal plants using CUPRAC method, and (2) to highlight similarities or differences between results for the antioxidant activity estimated by FRAP and CUPRAC (cupric ion reducing antioxidant capacity) methods. The medicinal herbs have been purchased from the local market. The antioxidant activity of medicinal plants by CUPRAC method ranged from 34.20 µmol of Trolox equivalents /g (Urtica dioica) and 512,48 µmol of Trolox equivalents /g (Melissae Folium). The antioxidant capacity values after the CUPRAC method have been very high compared to those determined by FRAP method. The six herbs which recorded the highest antioxidant capacity by FRAP method are also found in the group of plants with very high antioxidant activity according with the CUPRAC method. In both cases the lowest antioxidant activity was assessed for Urtica dioica. Also, the last four positions were occupied by the same plants (Chelidonium majus, Capsella bursa pastoris, Valeriana officinalis and Urtica dioica) both for FRAP method and for the CUPRAC method. The hierarchies of medicinal plants, according to their antioxidant activities, were different for the two methods of measuring, respectively CUPRAC and FRAP.

Keywords: antioxidants, methods, diseases, FRAP, CUPRAC

1. Introduction

Antioxidants from our diet plays an important role in helping endogenous antioxidants for the neutralization of oxidative stress. The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies. (J.K. Willcox & al. [1], I.Young & al. [2]).

There are a lot of scientific papers which have brought important evidences relating to the role of oxidative stress in cardiovascular diseases as atherosclerosis, hypertension, ischemia, cardiomyopathy, cardiac hypertrophy (T. Bahorun & al. [3], M. Chatterjee & al. [4], A. Ceriello [5]), neurological diseases - memory loss, depression, Parkinson's disease, Alzheimer's disease, multiple sclerosis, (B. Halliwell, [6], D.A. Butterfield [7]), nephropathy - glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia (J. Galle [8]), pulmonary diseases - asthma and chronic obstructive pulmonary disease (G. Caramori & al. [9], Y. Hoshino & al. [10], W. Macnee [11]), rheumatoid arthritis - (J. Walston & al. [12], C.H. Meyer & al [13]), ocular disease - cataracts (C.H. Meyer & al. [14], S. Beatty & al. [15]). Also, cancer initiation and promotion are induced by free radicals (M.

Valko & al. [16], Halliwell B [17]). The oxidative DNA damage is responsible for cancer development. (M. Valko & al. [16], M. Valko & al. [18]). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (A.P.H. Lien & al. [19]). Since they have so very big importance there are a large number of scientific researchers related to this field subject. Also, there are a lot of scientific papers about medicinal herbs. Sometimes, the published data for the same medicinal plants differ from one scientific researcher to the other. The differences could be caused by many factors (e.g. the extraction mod, the sample preparation, the origin of samples, etc.). Also, the methods used for antioxidant activity estimation could lead to the significant differences. Some methods measure only the hydrophilic antioxidants (like Folin and FRAP), while others detect only those soluble in organic solvents, especially alcohols (like DPPH) (M.B. Arnao [20]). Therefore, the aim of this study was to estimate the influence of different methods of measuring the antioxidant activity of the medicinal herbs.

2. 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 CUPRAC methods was made using SPECORD 205 spectrophotometer by Analytik Jena.

Plant material and sampling

In the present study, fifteen medicinal plants were analyzed for the antioxidant activity by CUPRAC method. The medicinal herbs were purchased from the local market. The results obtained using CUPRAC method were compared with the antioxidant activities of the same medicinal herbs, which were obtained by FRAP method (D.S. Stef & al. [21]).

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 the antioxidant activity by CUPRAC method

The method is named as “cupric reducing antioxidant capacity” abbreviated as the CUPRAC method since copper (II) (or cupric) ion reducing ability is measured (R. Apak & al. [22]).

The chromogenic redox reagent used for the CUPRAC assay was bis (neocuproine) copper (II) chelate. This reagent is useful at pH 7 and the absorbance of the Cu (I)-chelate formed as a result of redox reaction with reducing antioxidants was measured at 450 nm. The chromogenic oxidizing reagent of the developed CUPRAC method, i.e., bis (neocuproine) copper (II) chloride (Cu (II)-Nc), reacts with n-electron reductant antioxidants (AO) in the following manner:

$$n\ Cu(Nc)2^{2+} + n\text{-electron reductant (AO)} \leftrightarrow n\ Cu(Nc)2^{+} + n\text{-electron oxidized product} + n\ H^{+}$$

3. Results and discussion

The registered values for the antioxidant activity estimated by CUPRAC method are presented in table 1.

Table 1 The antioxidant activity estimated by CUPRAC [µmol of Trolox equivalents (TE)/g]

No	Specification	Mean ± SD	No	Specification	Mean ± SD
1	Hypericum perforatum	503.98 ±1.136	9	Symphytum officinale	277.26 ± 0.531
2	Tilia platyphyllos	476.32 ± 0.857	10	Galium mollugo	242.05 ± 0.533
3	Salix babylonica	476.09 ± 0.810	11	Poligonum aviculare	253.80 ± 0.489
4	Violae tricoloris herba	205.10 ± 0.421	12	Valeriana officinalis	154.02 ± 0.363
5	Melissae folium	512.48 ± 0.875	13	Chelidonium majus	141.94 ± 0.291
6	Epilobium montanum	510.24 ± 0.909	14	Capsella bursa pastoris	98.06 ± 0.360
7	Salvia officinalis	484.33 ± 0.730	15	Urtica dioica	34.20 ± 0.334
8	Plantago major	338.79 ± 0.633			

For statistical analysis of the obtained data the program IBM SPSS ANOVA with Tukey was used. The significant differences ($p < 0.001$) were registered between almost all variants, with the following exceptions: $p < 0.05$ between *Melissa folium* and *Epilobium montanum*; $p > 0.05$ (not significant) between *Tilia platyphyllos* and *Salix babylonica*.

The antioxidant activity of analized medicinal plants ranged between 34.20 µmol Trolox/g and 512,48 µmol of Trolox equivalents /g. According with their antioxidant activity these herbs could be divided into three groups.

The first group contains herbs with very high antioxidant capacity (*Melissae Folium, Epilobium montanum, Hypericum perforatum, Salvia officinalis, Salix babylonica* and *Tilia platyphyllos).* Their antioxidant activities varied between 476.09 and 512.48 µmol of Trolox equivalents /g.

The antioxidant activities of herbs from the second group ranged between 141.94 µmol of Trolox equivalents /g (*Chelidonium majus*) and 277.26 µmol of Trolox equivalents /g (*Symphytum officinale*).

The lowest values were noticed for *Capsella bursa pastoris* and *Urtica dioica* (98.06 and 34.2 µmol of Trolox equivalents /g).

The antioxidant activity (AA) was measured using two methods (FRAP (21) and CUPRAC) and the results of these two determinations were compared (figure 1). It was not expected to obtain identical or similarly results. Instead it was expected that the hierarchy of these results to be the same, or at least very close.

According with obtained data can be said that the values for AA-FRAP have ranged in a close interval (206.0 – 40.21 µmol of Trolox equivalents /g) compared with those of AA-CUPRAC (512.48 - 34.2 µmol of Trolox equivalents /g). Also, thirteen out of fifteen herbs have ranged between 145.0 – 206.0 µmol of Trolox equivalents /g.

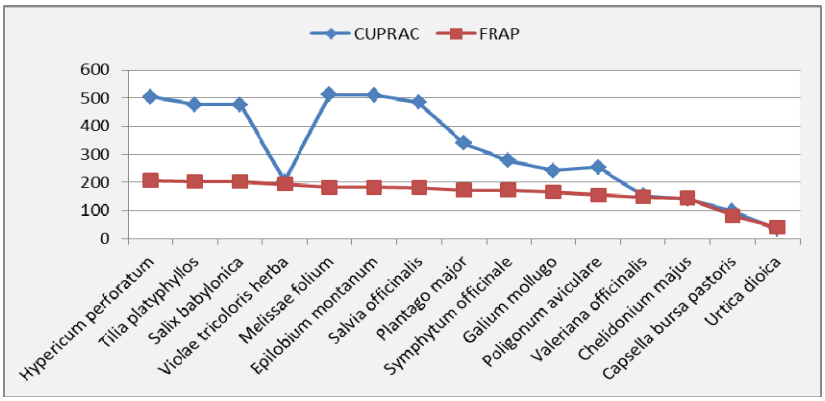

Figure 1 The hierarchies of fifteen medicinal plants
according with their antioxidant activities measured by CUPRAC and FRAP methods

In figure 1 can be seen that antioxidant activities measured with CUPRAC method have formed at least three distinct groups. Due to these reasons can be said that the hierarchies are very different for the antioxidant activities of the medicinal herbs which were measured by the FRAP and CUPRAC methods.

In figure 2 are shown the differences between methods for each of these fifteen medicinal plants.

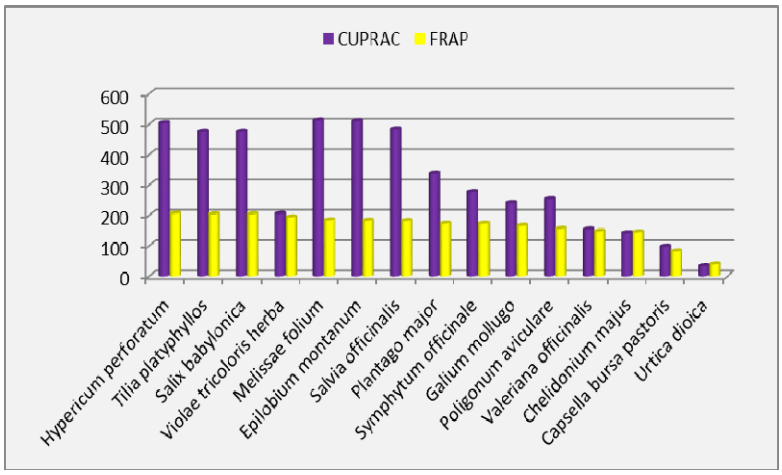

Figure 2 The differences among antioxidant activities of herbs measured by the CUPRAC and FRAP methods

The antioxidant activities for *Urtica dioica* and *Chelidonium majus* estimated by CUPRAC method have got lower values compared with the values obtained using FRAP method. All other medicinal herbs showed higher values for the antioxidant activity estimated by CUPRAC method.

The differences between AA from two methods were very large. There were some medicinal herbs which has the antioxidant activities by CUPRAC method higher than double compared with the values registered for FRAP method (e.g. *Melissae Folium, Epilobium montanum, Hypericum perforatum, Salvia officinalis, Salix babylonica* and *Tilia platyphyllos*).

There are different reasons that lead to these differences. One of these reasons could be that CUPRAC method can determine the antioxidant substances containing sulfhydryl group (SH), for example the proteins (S. Dragan & al. [23]). Not all methods measure protein-thiols, or smaller molecule -SH compounds of different origin (such as GSH, with FRAP. Also, The CUPRAC method is advantageous over FRAP since the redox chemistry of copper(II) -as opposed to that of chemically inert high-spin ferric ion having half-filled d-orbitals in its electronic configuration- should involve faster kinetics. The bis (neocuproine) copper (I) cation chromophore is soluble both in water and organic media; therefore the CUPRAC method is capable to assay both hydrophilic and lipophilic antioxidants. (D. Huang & al. [24]).

4. Conclusion

The results of this paper shown that using CUPRAC method for antioxidant activity of medicinal herbs resulted not only in different antioxidant activities of them, but also in changing of hierarchy for antioxidant activity (estimated by FRAP) of these medicinal plants.

The assessment of antioxidant capacity of medicinal plants is just the first step in research activity. Even so, it is very important to have accurate assessments and in future researches should bow of comparable data. If the preliminary data for *in vitro* or *in vivo* scientific researches are different, certainly the results of these researches will be different.

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