THE DETERMINATION OF TOTAL ANTIOXIDANT CAPACITY OF MEDICINAL PLANTS FROM ROMANIA BY NIR SPECTROSCOPY

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In this study are presented our researches regarding the determination of total antioxidant capacity of 17 medicinal plants from Romania using NIR Spectroscopy. For this it were realized correlations between the values obtained for total antioxidant capacity (mM/g) using chemical FRAP method and the reflectance values of frequencies from NIR spectra. To obtain the main parameters of the regression model for total antioxidant capacity determination using NIR Spectroscopy it was used PLS-Laverage method of UNSCRAMBLE software. The correlation coefficient R^2 obtained for regression equation was equal with 0.993230. The good quality of prediction of obtained PLS-Leverage model was done by the small values of the deviation, around 2-3%.

***Key words**: TAC - FRAP, NIR Spectroscopy, medicinal plants*

The free radicals that appear in human organism cause damage for two reasons. First reason is that free radicals are very reactive species in biochemical plan, which attack and destroy the cell membrane. The second reason is that free radicals attack permanent. Free radicals don't stop, having the properties to reproduce more and more [Cooper, 2004].

The consumption of medicinal plants in human diet is associated with the reducing of chronic diseases risks such as cancer and coronary heart disease. This protection against many diseases has been attributed to the various antioxidants contained in them. Antioxidants have the capacity to neutralize free radicals, which cause oxidative damage to biological molecules [Podsedek et al, 2003].

In our days the determination of total antioxidant capacity using FRAP method request a great consumption of reagents, a long time to perform the analyses and the qualified human resources. Near Infrared Reflectance Spectroscopy (NIR Spectroscopy) is a non-destructive and very quickly qualitative and quantitative analyze method [Wilson, 1994]. The necessity to study NIRS method applications in our country to characterize the total antioxidant capacity

refers both to calibrate this method for the plants species existed in different pedo-geographically zones from Romania and to organize a national database.

The main objective of this study was to determine the total antioxidant capacity (TAC) of some medicinal plants from Romania by Near Infrared Reflectance Spectroscopy. For this, medicinal plants samples were analyzed for total antioxidant capacity using both NIR Spectroscopy and chemical FRAP methods. Then it was made correlations between the values of reflectance obtained for TAC using NIR Spectroscopy with those obtained using chemical FRAP method.

MATERIAL AND METHODS

Samples preparation. It was analyzed 17 medicinal plants used currently in treatment of different diseases. The analyzed medicinal plants were dried both in normal condition and at 105^{o}C for 4 h. The analyzed medicinal plants were: *Epilobium hirsutum* (eng. – Great willowherb; rom. – Pufuliţă), *Arnica montana* (eng. – Mountain arnica; rom. – Arnică), *Ocimum basilicum* (eng. – Basil; rom. – Busuioc), *Rosmarinus officinalis* (eng. – Rosemary; rom. – Rozmarin), *Taraxacum officinale* (eng. – Dandelion; rom. – Păpădie), *Salvia officinalis* (eng. – Common sage; rom. – Salvie), *Silybum marianum* (eng. – Blessed Milk Thistle; rom. – Armurariu), *Althaea officinalis* (eng. – Marshmallow; rom. – Nalbă), *Rhamnus frangula* (eng. – Alder Buckthorn; rom. – Cruşin), *Echinacea* (eng. – Purple coneflower; rom. – Echinacea), *Chelidonium majus* (eng. – Greater celandine; rom. – Rostopască), *Melissa officinalis* (eng. – Lemon balm; rom. – Roiniţă), *Artemisia absinthium* (eng. – Absinthium; rom. – Pelin), *Foeniculum vulgare* (eng. – Fennel; rom. – Fenicul), *Plantago lanceolata* (eng. – Ribwort plantain; rom. - Pătlagină), *Crataegus monogyna* (eng. – Common hawthorn; rom. – Păducel) and *Hippophae rhamnoides* (eng. – Sea buckthorn; rom. – Cătină).

The hydro-etanolic extracts for FRAP determination were prepared as following: 1 g medicinal plant sample, dried both in normal atmospheric condition and at 105^{o}C for 4 h, grounded and sieved at 0.3 mm, was mixed with 50% ethanol 20 mL. The obtained hydro-etanolic extracts were rested for 30 minutes and filtered.

Determination of Total Antioxidant Capacity (TAC) by FRAP method

Total Antioxidant Capacity (mM/g) was determined chemically using FRAP method. FRAP method depend upon the reduction at low pH of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant (antioxidant). This ferrous tripyridyltriazine complex has an intensive blue colour and can be monitored at 593 nm [Benzie & Strain, 1996].

Reagents: acetate buffer, 300 mM/L, pH 3.6 (3.1g sodium acetate $3H_2O$ 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 $FeCl_36H_2O$ in distilled water. FRAP working solution: 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$ solution. It is necessary to prepare freshly the working solution.

Aqueous solution of known Fe (II) concentration was used for calibration, in a range of 0.1-0.6 mM/L. For the preparation of calibration curve 0.5 mL aliquot of 0.1, 0.2, 0.4, and 0.6 μM/mL aqueous Fe(II) as Mohr salts solution (1mM) were mixed with 2.5 mL FRAP working solution. FRAP reagent was used as blank [Gergen, 2004].

The absorption was read after 30 minutes at 25 °C and 593 nm. All solutions were prepared using deionized water. For all the samples the determination were made in triplicate and the mean values were reported. Total antioxidant capacity in Fe (II)

equivalents was calculated. Correlation coefficient (R^2) for calibration curve was 0.9979.

Absorption determination for TAC (FRAP) was made using UV-VIS spectrophotometer SPECORD 205 by Analytik Jena.

NIRS spectra were recorded with V 670 Spectrophotometer instrument by Abble-Jasco in the range 800-2500 nm. For all the samples the scan was made in duplicate.

Statistical interpretation of obtained data was performed with UNSCRAMBLE, a complex program for multivariate data analyses and interpretation. Using this statistical program it is possible to perform the variance, covariance and also the relevant correlations between different complex data matrix.

RESULTS AND DISCUSIONS

The values obtained for TAC (mM/g) for the analyzed medicinal plants samples, dried both in normal atmospheric condition and at 105^{o}C for 4 h, using FRAP method, are present in *tab. 1*.

Table 1

TAC (mM/g) for the analyzed medicinal plants samples, determinated using FRAP method

Samples	TAC (FRAP)-normal dried (mM/g)	TAC (FRAP)-dried at 105^{o}C (mM/g)
1. Epilobium hirsutum	134.00	132.20
2. Arnica montana	129.20	128.20
3. Ocimum basilicum	128.40	127.80
4. Rosmarinus officinalis	131.60	130.20
5. Taraxacum officinale	121.80	125.40
6. Salvia officinalis	129.80	128.80
7. Silybum marianum	31.20	36.20
8. Althaea officinalis	58.60	67.60
9. Rhamnus frangula	116.60	117.00
10. Echinacea	95.40	108.40
11. Chelidonium majus	120.40	125.20
12. Melissa officinalis	134.00	130.80
13. Artemisia absinthium	104.4	115.20
14. Foeniculum vulgare	91.80	94.80
15. Plantago lanceolata	127.60	126.60
16. Crataegus monogyna	128.40	127.20
17. Hippophae rhamnoides	125.20	126.40

To obtain the main parameters of the regression model for total antioxidant capacity determination using NIR Spectroscopy it was used PLS-Laverage method of UNSCRAMBLE software. The graphical representation of correlation between the values for TAC (mM/g) obtained using FRAP method and predicted values by regression equation on the basis of reflectance from NIR spectra for all analyzed samples is presented in *fig. 1*.

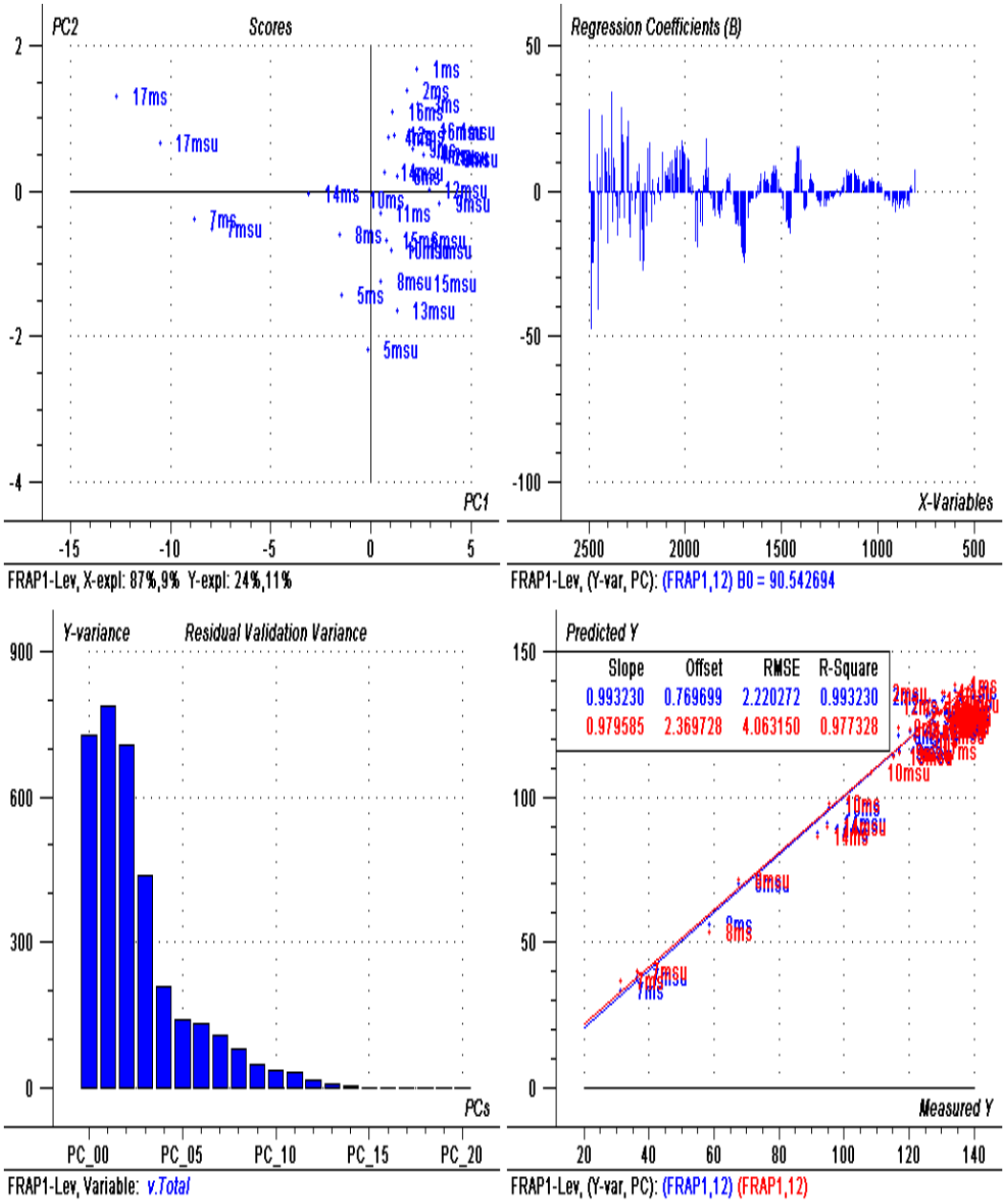

Figure 1 **Correlation between the values for TAC (mM/g) obtained using FRAPS method for all analyzed samples and predicted values by regression equation on the basis of reflectance values from NIR spectra (PLS-Laverage method)**

The main parameter of the PLS-Laverage model is the correlation coefficient of regression equation (R^2), which must be around 1 for a good correlation between the interpreted data.

The PLS-Laverage model present also both the variation of this coefficient for the entire analyzed spectral domain and the dependence of variance for the main component. The variation of regression coefficient decreased quickly after the main ten components, which mean a good functionality of the model.

The graphical representation of the scores for the main two components shows the distribution of the analyzed samples depending on the model variables. The grouping of the samples in one quadrate of xy-axis means a convergent model, with a good prediction of the results.

In our case the correlation coefficient for regression equation between the values for total antioxidant capacity determinated by FRAP method and those for reflectance from NIR spectra was very good, the variance was small, the number of main components was also reduce at 5. The groping of the analyzed samples in one quadrate of xy-axis depending of the main two components means a good quality of the model.

To test the precision of the prediction of obtained PLS-Leverage model it was selected aleatory ten medicinal plants, five plants dried in normal condition and five plants dried at 105^{o}C for 4 h, and was scanned the NIR spectra. Then, using the reflectance values from NIR spectra and obtained PLS-Leverage model were predicted the values of total antioxidant capacity for the selected medicinal plants (*tab. 2 and fig. 2*). The obtained results were compared with those determinated by FRAP method.

Table 2

The predicted values of total antioxidant capacity for the selected five medicinal plants using NIR spectra and obtained PLS-Leverage model

Samples	Predected values for TAC (FRAP) (mM/g)	Real values for TAC (FRAP) (mM/g)	Deviation values (%)
1. Epilobium hirsutum	136.31	134.00	2.82
2. Arnica montana	128.78	129.20	2.59
3. Ocimum basilicum	128.92	128.40	2.25
4. Rosmarinus officinalis	128.82	131.60	2.48
5. Taraxacum officinale	120.71	121.80	2.48
13. Artemisia absinthium	114.26	115.20	2.40
14. Foeniculum vulgare	91.18	94.80	2.52
15. Plantago lanceolata	125.17	126.60	2.22
16. Crataegus monogyna	127.16	127.20	2.51
17. Hippophae rhamnoides	128.21	126.405	2.90

It is possible to observe the good quality of prediction for obtained PLS-Leverage model analyzing the small values of the deviation, around 2-3%.

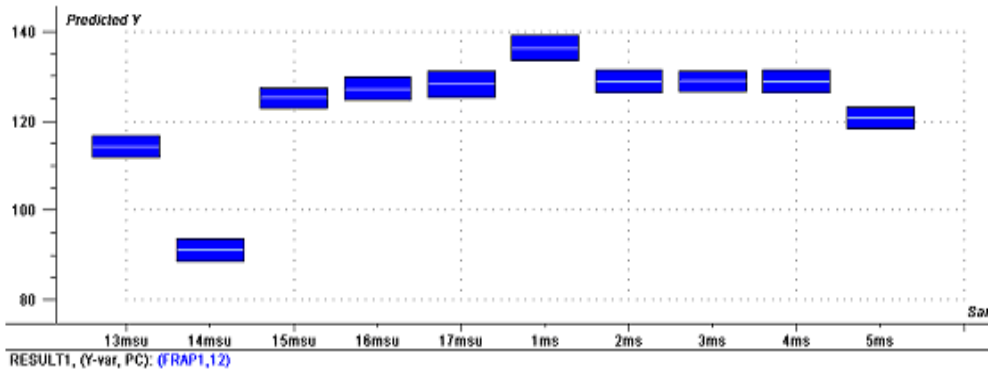

Figure 2 **The graphical representation of predicted values of total antioxidant capacity for the selected five medicinal plants using NIR spectra and obtained PLS-Leverage model**

The promising results obtained encourage us to continue the study using a lot of samples to obtain superior statistical parameters for regression equation between TAC (FRAP) and the values of reflectance from NIR spectra.

CONCLUSIONS

The obtained results, using a small number of samples (34), shows a good prediction for the total antioxidant capacity using PLS-Leverage model, constructed with the values determinated by FRAP method and those for reflectance from NIR spectra.

The good prediction obtained for TAC demonstrates that NIR spectra contain o lot of information about some chemical structures of organic compounds with antioxidant capacity.

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