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# The influence of level and source of microelements on the antioxidant activity of medicinal herbs

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**ŞTEF D. S., GERGEN I., TRAŞCĂ T. I., ŞTEF LAVINIA, RIVIS A., HEGHEDUŞ M. G., BORDEAN DESPINA**
*Banat University of Agricultural Sciences and Veterinary Medicine, 119 Aradului Street, Timsoara, Romania, e-mail:ducu_stef@yahoo.com, tel. 0720687239*

## Abstract

*The aim of this paper was to analyze the influence of microelements source and composition on the antioxidant activity of medicinal herbs. The microelements administered through salts, at NRC (Nutrient Requirements Council) level (A) increased significantly $TAC_{FRAP}$ (total antioxidant capacity by FRAP method) for all groups of medicinal plants (a, b and c). The mineral salts and fritta improved $TAC_{FRAP}$ in the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percent differences were: +15.61 for A, +9.3 for B, +6.34 for C and D. Additionally, mineral salts improved $TAC_{FRAP}$ for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP}$. The lower values were recorded on $TAC_{FRAP}$ for the groups (b) and (c) when microelements were provided via fritta and Sel-Plex. The mineral salts and fritta improved total phenols in the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percent differences were: +9.03 for A, +5.46 for B, +2.45 for C and D. Also mineral salts improved the total phenols for the each group of medicinal herbs with medium (b) and low (c) $TAC_{FRAP}$. The lower values on total phenols were obtained for the groups (b) and (c) when microelements were provided via fritta and Sel-Plex. The results noticed at 24 hours were similarly for both and differences were quite similarly with the first estimation.*

**Keywords:** *antioxidant capacities, FRAP method, total phenols, DPPH, medicinal herbs*

## Introduction

Phenolic compounds are secondary plant metabolites that are naturally present in almost all plant materials, including food products of plant origin. These compounds are thought to be an integral part of both human and animal diets. They can enhance the oxidation stability of oil, especially in olive oil (Psomiadou and Tsimidou, 2002[1]).

Since they exhibit antioxidative properties, they have attracted considerable interest. It has been reported that they have antimutagenic and anticarcinogenic (Kampa et al., 2004[2]), cardioprotective (Caccetta et al. 2000[3]), and antimicrobial properties (Friedman and Jurgens, 2000[4]; Wen et al. 2003[5]).

Transition metals have a major role in the generation of oxygen free radicals in living organisms. Iron exists in two distinct oxidation states - ferrous and ferric ions. The ferric ion ($Fe^{3+}$) is the relatively biologically inactive form of iron. However, it can be reduced to the active $Fe^{2+}$, depending on the conditions, particularly pH (Strlic et al. 2002[6]), and oxidized back through Fenton type reactions, with production of hydroxyl radicals or Haber-Weiss Cycle reactions with superoxide anions (Wong and Kitts, 2001[7]). The production of these radicals can lead to lipid peroxidation, protein modification and DNA damage. Chelating agents may inactivate metal ions and potentially inhibit the metal-dependent processes (Finefrock et al. 2003[8]).

There are some ambiguous results in literature concerning metal-chelation properties of polyphenols. Since they may act as antioxidants and pro-oxidants this may lead to a reduction in their antioxidant properties (Keceli and Gordon, 2002[9]; Moran et al. 1997[10]). Metal chelation is

recognized by some authors as a minor mechanism in some polyphenols (Rice-Evans et al. 1996[11]), yet the contribution of free radical scavenging or of metal ion chelation to the anti-oxidative effect of polyphenols is not fully specified (Sugihara et al. 2001[12]). The metal chelating ability of polyphenols is related to the presence of ortho-dihydroxy polyphenols, i.e., molecules bearing catechol or galloyl groups (Khokhar and Apen-ten, 2003[13]; Moran et al. 1997[14]). Since metal chelation can occur at physiological pH it has a physiological significance.

The aim of this paper was to analyze the influence of microelements content and source on the antioxidant activity of medicinal herbs.

## Materials and methods

### Reagents and equipment

All chemicals and reagents were analytical grade or purest quality and purchased from Sigma-Aldrich, Merck 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 15 mixtures of medicinal herbs from local markets and microelements were analyzed for total antioxidant capacity. The medicinal herbs used in this trial were chosen according to their total antioxidant capacity: the first group (a) had a very high total phenols (783-1168 µmol Trolox/g) and $TAC_{FRAP}$ (182-206 µmol Trolox/g); the second group (b) had a medium total phenols (349-586 µmol Trolox/g) and $TAC_{FRAP}$ (130-199 µmol Trolox/g); the third group (c) had a very low total phenols (<160 µmol Trolox/g) and $TAC_{FRAP}$ (<60 µmol Trolox/g). The mineral salts and fritta were the two sources of microelements. Both medicinal herbs and microelements used in this experiment are presented in table 1.

**Table 1.** The experimental design

| Specification | A | B | C | D |
|---|---|---|---|---|
| **(a) -** Very high total antioxidant capacity *Epilobium montanum, Melissae Folium, Hypericum perforatum, Salvia officinalis, Crataegus monogyna* 1 | (a) + A 2 | (a) + B 3 | (a) + C 4 | (a) +D 5 |
| **(b) -** Medium total antioxidant capacity *Thy mi herba, Rhamnus frangula, Plantago major, Mentha piperita, Hippophae rhamnoides* 6 | (b)+ A 7 | (b)+ B 8 | (b)+ C 9 | (b)+ D 10 |
| **(c) -** Low total antioxidant capacity *Phoeniculus, Urtica dioica. Artemisia absinthium, Cynara scolymus, Malva silvestris* 11 | (c)+A 12 | (c)+ B 13 | (c)+ C 14 | (c)+ D 15 |

**A -** Microelements were provided with salts, at NRC level (80 mg/kg Fe, 60 mg/kg Mn, 40 mg/kg Zn, 8 mg/kg Cu, 0.15 mg/kg Se and 0.35 mg/kg Co)

**B** - Microelements were provided with salts, at the level of 1g fritta (42,76 mg/kg Fe, 59,1 mg/kg Mn, 53,3 mg/kg Zn, 4,57 mg/kg Cu, 0,15 mg/kg Se and 0,626 mg/kg Co)

**C -** Microelements were provided with fritta: 1g and Se by Sel-Plex (42,76 mg/kg Fe, 59,1 mg/kg Mn, 53,3 mg/kg Zn, 4,57 mg/kg Cu, 0,15 mg/kg Se and 0,626 mg/kg Co)

**D** - Microelements were provided with fritta: 0,35g and Se by Sel-Plex (14,97 mg/kg Fe, 20,68 mg/kg Mn, 18,65 mg/kg Zn, 1,60 mg/kg Cu, 0,15 mg/kg Se and 0,22 mg/kg Co)

(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

For the antioxidant extraction ethanolic (50%) extracts in ratio 10/20 were prepared. 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 depends 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[15]].

Reagents: acetate buffer, 300mM/L, pH 3.6 (3.1g sodium acetate $3H_2O$ and 16 mL 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. 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 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. 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.

**The amount of polyphenolic compounds**

The following reagents were used: 2.0 M Folin-Ciocalteu phenol reagent, Trolox and anhydrous carbonate. The total content of polyphenolic compounds in the medicinal herbs and microelements mixture was determined from the ethanol extracts diluted 1/10 using the Folin-Ciocalteu method (1927). For the preparation of the calibration curve 0.5 mL aliquot of 0.2, 0.3, 0.4, 0.8 and 1.2 μM/mL aqueous Trolox solution were mixed with 2.5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2.0 mL sodium carbonate solution (7.5%). The absorption was read after 2 h at 20°C, at 750 nm. All measurements were performed in triplicate. Total content of polyphenols in samples in Trolox equivalents was calculated. Squared correlation coefficient ($r^2$) for calibration curve was 0.9883.

## Results and Discussion

The results for total antioxidant capacity $TAC_{FRAP}$ and total phenols of mixtures by medicinal herbs and microelements are presented in Table 2.

**Table 2.** Total antioxidant capacity $TAC_{FRAP}$ and polyphenols

| Specification | FRAP [μmol Trolox/g] | Total phenols [μmol Trolox/g] | FRAP 2* [μmol Trolox/g] | Total phenols 2* [μmol Trolox/g] |
|---|---|---|---|---|
| **0** | **1** | **2** | **3** | **4** |
| 1 (a) | 205 | 531 | 217.6 | 518,4 |
| 2 (a + A) | 237 | 579 | 217.6 | 464 |
| 3 (a + B) | 224 | 560 | 214.8 | 464 |
| 4 (a + C) | 218 | 544 | 208 | 534,4 |
| 5 (a + D) | 218 | 544 | 201.6 | 534,4 |
| 6 (b) | 138 | 301 | 118.4 | 300,8 |
| 7 (b+A) | 150 | 323 | 137.6 | 300,8 |
| 8 (b+B) | 144 | 314 | 128 | 310,4 |
| 9 (b+C) | 131 | 288 | 118.4 | 272 |
| 10 (b+D) | 122 | 282 | 115.2 | 259,2 |
| 11 (c) | 35 | 83 | 25.6 | 76,8 |

| 0 | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 12 (c+A) | 58 | 118 | 48 | 92,8 |
| 13 (c+B) | 42 | 93 | 32 | 96 |
| 14 (c+C) | 29 | 83 | 22.4 | 124,8 |
| 15 (c+D) | 22 | 64 | 22.4 | 76,8 |

*the estimations were made after 24 hours mixtures keeping

## The influence of microelements level and source on $TAC_{FRAP}$ of medicinal herbs

Automatic data processing was performed using cluster analysis. The results are presented in tables 3-5 and figure 1.

**Table 3.** The results for $TAC_{FRAP}$ [µmol Trolox/g]

| Specification | X | A | B | C | D |
|---|---|---|---|---|---|
| a | 205 | 237 | 224 | 218 | 218 |
| b | 138 | 150 | 144 | 131 | 122 |
| c | 35 | 58 | 42 | 29 | 22 |

**Table 4**. Transformed data

| | X | A | B | C | D |
|---|---|---|---|---|---|
| a | 7,687 | 7,895 | 7,814 | 7,775 | 7,775 |
| b | 7,119 | 7,238 | 7,180 | 7,044 | 6,943 |
| c | 5,170 | 5,883 | 5,426 | 4,907 | 4,524 |

**Table 5.** Distance matrix

| | X | A | B | C | D |
|---|---|---|---|---|---|
| X | 0,000 | | | | |
| A | **0,145** | 0,000 | | | |
| B | 0,055 | 0,091 | 0,000 | | |
| C | 0,055 | 0,195 | 0,105 | 0,000 | |
| D | **0,131** | 0,272 | 0,182 | 0,077 | 0,000 |

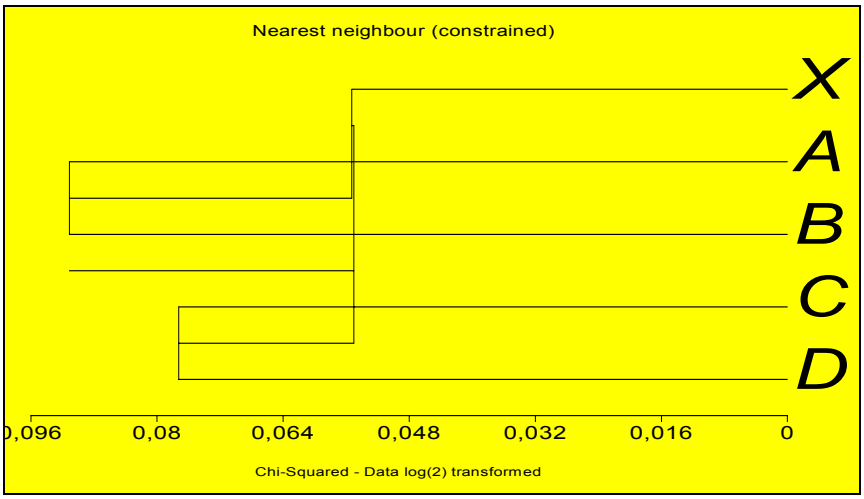


**Figure 1.** The nearest neighbour for $TAC_{FRAP}$ (after 10 min.)

According with the cluster analysis it can be concluded that:

- microelements provided via salts, at NRC level (A) increased significantly $TAC_{FRAP}$ for all groups of medicinal plants (a, b and c);

- the groups A and B which had microelements provided through mineral salts act similarly (matrix distance - 0.077); also the groups C and D which had microelements provided via fritta and Sel-Plex act similarly (matrix distance -0.091);

According with this results it can be concluded that both mineral salts and fritta improve $TAC_{FRAP}$ for the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percent differences were: +15.61 for A, +9.3 for B, +6.34 for C and D. Also mineral salts improve $TAC_{FRAP}$ for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP}$. The lower values were recorded on $TAC_{FRAP}$ for the groups (b) and (c) when microelements were provided via fritta and Sel-Plex.

The results for $TAC_{FRAP}$ at 24 hours are presented in table 6 and in figure 2.

**Table 6.** The results for $TAC_{FRAP}$ [µmol Trolox/g]

| Specification | X | A | B | C | D |
|---|---|---|---|---|---|
| a | 217,6 | 217,6 | 214,8 | 208 | 201,6 |
| b | 118,4 | 137,6 | 128 | 118,4 | 115,2 |
| c | 25,6 | 48 | 32 | 22,4 | 22,4 |

The higher $TAC_{FRAP}$ values are obtained when microelements were provided through mineral salts at NRC level (+16.2 for b and +87.5 for c), followed by the group which had microelements given through mineral salts at the level of 1g fritta (+8.1 for b and +28 for c).

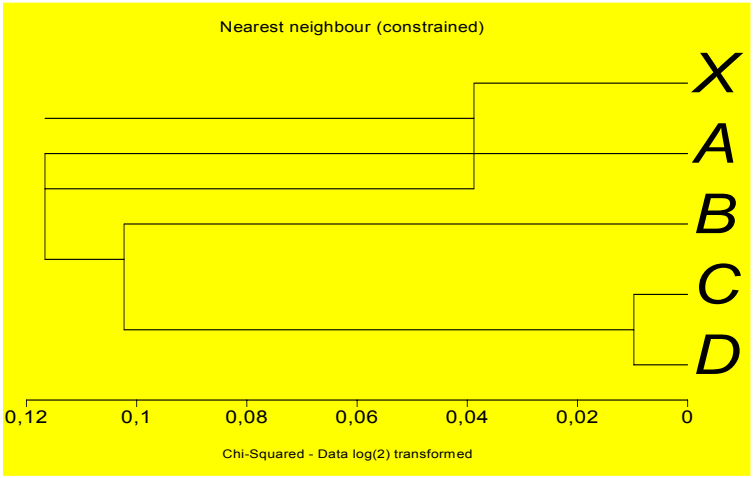


**Figure 2.** The nearest neighbour for $TAC_{FRAP}$ after 24 hours

The $TAC_{FRAP}$ values are smaller for all medicinal plants group when microelements were ensured with fritta and Sel-Plex.

## The influence of level and source of microelements on the total phenols of the medicinal herbs

The results for total phenols of mixtures by medicinal herbs and microelements are presented in table 7 and figure 3.

**Table 7.** The results for total phenols at 1 hour [µmol Trolox/g]

| Specification | X | A | B | C | D |
|---|---|---|---|---|---|
| a | 531 | 579 | 560 | 544 | 544 |
| b | 301 | 323 | 314 | 288 | 282 |
| c | 83 | 118 | 93 | 83 | 64 |

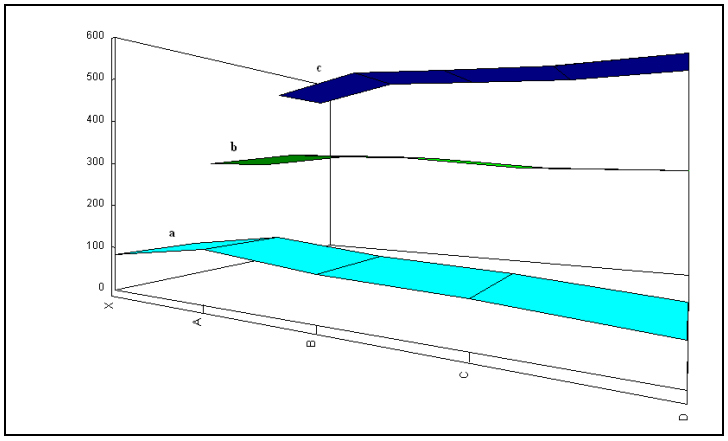


**Figure 3.** The total phenols of experimental variants

Both mineral salts and fritta improve total phenols for the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percent differences were: +9.03 for A, +5.46 for B, +2.45 for C and D. Also mineral salts improve total phenols for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP.}$ The lower values were registered on total phenols for the groups (b) and (c) when microelements were ensured with the fritta and Sel-Plex.

The results for total phenols at 24 hours are presented in table 8.

**Table 8.** The results for total phenols at 24 hour [μmol Trolox/g]

| Specification | X | A | B | C | D |
|---|---|---|---|---|---|
| a | 518,4 | 464 | 464 | 534,4 | 534,4 |
| b | 300,8 | 300,8 | 310,4 | 272 | 259,2 |
| c | 76,8 | 92,8 | 96 | 124,8 | 76,8 |

The group (a) was obtained the best results for total phenols at 24 hours in C and D experimental variants. The percentual difference was +3.08. Total phenols in (a) decreased for A and B (microelements were supplied from mineral salts) with -10.5.

The mineral salts improve total phenols for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP.}$ The lower values were recorded on total phenols for the group (b) when microelements were supplied from fritta and Sel-Plex.

For the group (c) and microelements supplied from fritta and Sel-Plex two conclusions can be outlined:

- similarly amounts (76.8 μmol Trolox/g) of total phenols were noticed for the experimental variants X and D;

- the others experimental variants registered higher values comparatively with X; the best result was obtained for the experimental variant C (+62.5), when microelements were ensured with fritta (1g) and Sel-Plex; also, the mineral salts (A and B) improve the total phenols at 24h.

## Conclusions

The microelements provided through salts, at NRC level (A) increased significantly $TAC_{FRAP}$ for all groups of medicinal plants (a, b and c). The mineral salts and fritta improve $TAC_{FRAP}$ for the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percentual differences were: +15.61 for A, +9.3 for B, +6.34 for C and D. Also mineral salts improve $TAC_{FRAP}$ for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP.}$ The lower

values were registered on $TAC_{FRAP}$ for the groups (b) and (c) when microelements were give through the fritta and Sel-Plex.

The mineral salts and fritta improve total phenols for the first group (a – very high $TAC_{FRAP}$) of the medicinal plants. The percent differences were: +9.03 for A, +5.46 for B, +2.45 for C and D. Also mineral salts improve total phenols for the groups of medicinal herbs with medium (b) and low (c) $TAC_{FRAP}$. The lower values were registered on total phenols for the groups (b) and (c) when microelements were provided via fritta and Sel-Plex.

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