# Evaluation of *in Vitro* Reducing Effect of Several Vegetable Extracts on the Digestive Bioavailability of Heavy Metals

**MIHAI APOSTU, GLADIOLA TANTARU, MADALINA VIERIU*, ALINA DIANA PANAINTE, NELA BIBIRE, LUMINITA AGOROAEI**
Grigore T. Popa University of Medicine and Pharmacy Iasi, Faculty of Pharmacy, 16th University Str.,700115, Iasi, Romania

*The study aimed at assessing the in vitro digestive bioavailability of heavy metals in the presence of plant extracts under simulated digestive system conditions. The complexing ability of aqueous herbal extracts of Crataegus sp., Tilia spp., Rosa canina, Vaccinium myrtillus, Geranium robertianum, Mentha piperita, Cynara cardunculus subsp. Scolymus, Plantago sp., and Coriandrum sativum was researched on $Cu^{+2}$, $Cd^{+2}$, $Ni^{+2}$, $Pb^{+2}$ and $Hg^{+2}$ cations. The quantitative determination of the fraction of free metal left, available for absorption, was achieved by atomic absorption spectroscopy (AAS) or potentiometry using an ion-selective electrode (ISE).*

*Keywords: heavy metals, digestive bioavailability, ISE, AAS.*

Heavy metals may enter the body via the oral route, inhalation, or dermal absorption and their toxicity manifests in the central and peripheral nervous systems, gastrointestinal, hematopoetic, renal, cardiovascular, and to a lesser extent in the musculoskeletal and reproductive systems [1-5]. Poisoning treatment aims to chelate the metal followed by biochemical transformation of the formed complex in a metabolite easy to remove through the urinary or bilio-faecal routes.

Recent studies have shown that plant products rich in polyphenols, tannins, polysaccharides and anthocyanins may inhibit the absorption of heavy metals in the body and promoting their elimination. Research conducted on herbs with a high polyphenols content have shown their high capacity to bind and facilitate removal of heavy metals [6-8]. Constant consumption of black tea can decrease the amount of absorbed Hg, derived from oceanic fish [9].

Assessing the ability of aqueous extracts of local herbs, to bind and increase the elimination of $Cu^{+2}$, $Cd^{+2}$, $Ni^{+2}$, $Pb^{+2}$, and $Hg^{+2}$ has required the development and validation of quantitative determination methods through atomic absorption spectroscopy [10] or potentiometry using selective membrane electrodes [11].

## Experimental part

The evaluation of the complexing capacity of the vegetable extracts has been done by treating an accurately measured volume of standard solution for each studied cation with an accurately measured volume of plant extract. The complexing ability of the extract was given by the difference between the initial concentration and final concentration of the free cation.

### *Material and methods*

The potentiometric measurements were made using a Hanna 301 digital pH/ millivoltmeter and a selective membrane electrode of our own construction. The atomic absorption spectrophotometry studies were made using a Analytik Jena ContrAA 300 spectrophotometer provided with air-acetylene flame atomizer and a high resolution continuous source. The operating parameters had been optimized in accordance with the manufacturer's recommendations. All reagents used were analytical grade.

## Results and discussions

### *Estimating the concentration of $Cu^{+2}$, $Cd^{+2}$, $Ni^{+2}$, $Pb^{+2}$, and $Hg^{+2}$ cations in the studied plant species and their aqueous extracts*

Plant species subjected to this study were analyzed to determine the concentration of existing heavy metals in the plant material used and their ability to release the cations during aqueous extraction. The studies were conducted comparatively through the two previously validated methods: AAS and ISE.

*The digestion of the vegetable material:* 6g of plant material was treated with 10mL of concentrated $HNO_3$ and allow to rest for 24 h. The mixture was heated in a water bath until all the nitrous vapor emission stopped. 4mL of $HClO_4$ 70% was added and the mixture was heated to yield a smaller volume that was filtered and diluted to 25mL with double distilled water.

*Preparation of infusions:* 6g of dry plant were added to 100mL of boiling double distilled water. After 30 min of rest, the mixture was filtered and diluted to 100mL with double distilled water.

*Digestion of infusions:* 100mL of infusion was heated in a water bath to yield a concnetrate. 5mL of $HNO_3$ and $HClO_4$ (5:1) mixture are added and it was all digested for two hours at 200°C in a Kjeldahl device. The product was filtered and diluted to 25mL with double distilled water.

The results obtained for *Crataegus sp.* (**CrS**), *Tilia sp.* (**TS**), *Rosa canina* (**RC**), *Vaccinium myrtillus* (**VM**), *Geranium robertianum* (**GR**), *Mentha piperita* (**MP**), *Cynara cardunculus subsp. Scolymus* (**CC**), *Plantago sp.* (**PS**) and *Coriandrum sativum* (**CoS**) are shown in table 1.

A comparative analysis of the results revealed a large variation in the heavy metal content of the dried plant material. $Cu^{+2}$ was predominant in all samples at various concentrations, while $Hg^{+2}$ was absent in all samples. Also the transfer of metal content from plant material to brewing varied from sample to sample. The $Cu^{+2}$ and $Ni^{+2}$ transfer occurred in various proportions while for $Cd^{+2}$ and $Pb^{+2}$ any amount transferred could not be assed through the analytical methods applied. There were significant differences between the results obtained through AAS and ISE.

* *email: vieriu_madalina@yahoo.com*

| Plant species | | Total concentrations of heavy metal cations (ppm, averages, n = 3) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Cu^{+2}$ | | $Cd^{+2}$ | | $Ni^{+2}$ | | $Pb^{+2}$ | | $Hg^{+2}$ | |
| | | I | II | I | II | I | II | I | II | I | II |
| CrS | AAS | 7.631 | 0.982 | 0.150 | - | 0.712 | 0.063 | 0500 | - | - | - |
| | ISE | 7.592 | 0.971 | 0.144 | - | 0.683 | 0.57 | 0482 | - | - | - |
| TS | AAS | 8.014 | 1.406 | 0.091 | - | 0.809 | 0.115 | - | - | - | - |
| | ISE | 7.984 | 1.390 | 0.72 | - | 0.781 | 0.111 | - | - | - | - |
| RC | AAS | 1.943 | 0.142 | 0.114 | - | 0.915 | 0.053 | - | - | - | - |
| | ISE | 1.910 | 0.140 | 0.091 | - | 0.890 | 0.049 | 0.161 | - | - | - |
| VM | AAS | 4.313 | 0.472 | 0.26 | - | 0.394 | 0.031 | 0.140 | - | - | - |
| | ISE | 4.303 | 0.460 | 0.018 | - | 0.373 | 0.029 | - | - | - | - |
| GR | AAS | 9.511 | 0.970 | 0.128 | - | 0.681 | 0.061 | - | - | - | - |
| | ISE | 9.482 | 0.966 | 0.100 | - | 0.672 | 0.057 | 0.108 | - | - | - |
| MP | AAS | 8.154 | 1.022 | 0.101 | - | 1.801 | 0.190 | 0.081 | - | - | - |
| | ISE | 8.115 | 1.013 | 0.090 | - | 1.771 | 0.187 | 0.610 | - | - | - |
| CC | AAS | 6.737 | 3.762 | - | - | 0.919 | 0.387 | 0.592 | - | - | - |
| | ISE | 6.608 | 3.695 | - | - | 0.890 | 0.382 | - | - | - | - |
| PS | AAS | 6.225 | 1.801 | - | - | 0.501 | 0.162 | - | - | - | - |
| | ISE | 6.013 | 1.742 | - | - | 0.470 | 0.145 | | - | - | - |
| CoS | AAS | 0.862 | 0.320 | 0.085 | - | 0.411 | 0.123 | 0.132 | - | - | - |
| | ISE | 0.764 | 0.286 | 0.079 | - | 0.381 | 0.110 | 0.128 | - | - | - |

**Table 1**
TOTAL CONCENTRATIONS OF HEAVY METAL CATIONS IN DRIED PLANT MATERIAL (I) AND INFUSION (II) AS mg/kg AND mg/L (ppm), RESPECTIVELY

*Correlations between chemical composition and heavy metal complexing effect*

Table 2 organizes the studied plant species based on the groups of active principles with chelating potential.

The possible mechanism of action is the inclusion of the metal into the structure of a complex (based on increased availability to polyvalent metals of hydroxyl, carbonyl, and carboxyl groups and also conjugated double bonds), thus its hydrophilic character increases so that it will not be absorbed, when it reaches the kidney it will be eliminated, and its potential for accumulation in the body will decrease.

**Table 2**
SYSTEMATIZATION OF PLANT SPECIES ACCORDING TO THE ACTIVE PRINCIPLES WITH CHELATING POTENTIAL

| Plant species | Active principles |
|---|---|
| *Crataegus sp.*<br>*Coriandrum sativum,*<br>*Cynara cardunculus ssp. Scolymus* | poliphenols |
| *Geranium robertianum*<br>*Mentha piperita,*<br>*Rosa canina* | tannins |
| *Tilia sp.*<br>*Plantago sp.* | polysaccharides |
| *Vaccinium myrtillus* | anthocyanins |

*Assessing the complexing capacity of the prepared extractive solutions for $Cu^{+2}$, $Cd^{+2}$, $Ni^{+2}$, $Pb^{+2}$,* and *$Hg^{+2}$ cations*

A standard solution of each cation was prepared in 100mL infusion (6g of dried plant/100mL) adjusted to *p*H 7.0 with NaOH, and the amount of free metal was determined. Because no precipitation occurred when the extracts were added, all quantifications of cations were performed using ISE. The complexing ability of the extract was established by the difference between the initial cation concentration and the concentration of the free cation that was determined using selective membrane electrodes (table 3).

To eliminate the interfering effect of free heavy metal natural content of the plant material that is transferred to the infusions, determinations were carried out on aqueous extracts. The results showed that the free cations concentration of infusions was not detectable through the used method, as opposed to the extract prepared by digestion when the determinations were done for the total quantity of all forms in which the heavy metal was released from plant material (free and various combination forms).

**Table 3**
ASSESSMENT OF COMPLEXING CAPACITY OF THE EXTRACTIVE SOLUTIONS FOR $Cu^{+2}$, $Cd^{+2}$, $Ni^{+2}$, $Pb^{+2}$ AND $Hg^{+2}$ CATIONS

| Plant species | Complexed metal concentration (µg/100mL infusion) (n = 3) | | | | |
|---|---|---|---|---|---|
| | $Cu^{+2}$ | $Cd^{+2}$ | $Ni^{+2}$ | $Pb^{+2}$ | $Hg^{+2}$ |
| RC | 3.14 ± 0.10 | 5.61 ± 0.17 | 3.01 ± 0.05 | 7.51 ± 0.12 | 6.53 ± 0.13 |
| MP | 5.17 ± 0.16 | 8.34 ± 0.26 | 4.86 ± 0.09 | 13.10 ± 0.22 | 12.87 ± 0.26 |
| GR | 7.83 ± 0.25 | 14.01 ± 0.44 | 6.93 ± 0.16 | 18.21 ± 0.30 | 17.52 ± 0.36 |
| VM | 10.11 ± 0.32 | 19.96 ± 0.63 | 9.77 ± 0.18 | 26.15 ± 0.44 | 24.01 ± 0.49 |
| CrS | - | - | - | - | - |
| TS | - | - | - | - | - |
| CC | - | - | - | - | - |
| PS | - | - | - | - | - |
| CoS | - | - | - | - | - |

**Table 4**
ASSESSMENT OF COMPLEXING CAPACITY OF THE EXTRACTIVE SOLUTIONS UNDER SIMULATED DIGESTIVE SYSTEM CONDITIONS

| Plant species | Complexed metal concentration (µg/100mL infusion) (n = 3) | | | | |
|---|---|---|---|---|---|
| | $Cu^{+2}$ | $Cd^{+2}$ | $Ni^{+2}$ | $Pb^{+2}$ | $Hg^{+2}$ |
| VM | 8.23 ± 0.11 | - | - | 1.47 ± 0.04 | 1.38 ± 0.02 |
| RC | - | - | - | | - |
| MP | - | - | - | | - |
| GR | - | - | - | | - |

| Total polyphenols mg GAE/100mL extract | Total flavonoids mg QE/100mL extract | Total anthocyanins mg C3GE/100mL extract |
|---|---|---|
| 15.28 ± 0.74 | 2.51 ± 0.19 | 5.60 ± 0.44 |

**Table 5**
TOTAL POLYPHENOLS, TOTAL FLAVONOIDS AND TOTAL ANTHOCYANINS CONTENT OF AQUEOUS EXTRACT OF *VACCINIUM MYRTILLUS* (n = 3)

Of the aqueous extracts tested, those of *Rosa canina*, *Mentha piperita*, *Geranium robertianum* and *Vaccinium myrtillus,* exhibited measurable complexing capacity for the cations analyzed, while infusions prepared from *Crataegus sp.*, *Tilia sp.*, *Cynara cardunculus subsp. Scolymus*, *Plantago sp.* and *Coriandrum sativum* proved to have no such capacity.

*Study of efficiency of extractive solutions under simulated digestive system conditions*

The infusions that proved to have complexing capacity during previous determinations were studied in the next phase under simulated digestive system conditions to quantify the bioavailability of ingested heavy metals in the presence of those extracts [9]. To that end, a standard solution of the studied cation was prepare in 100mL infusion (6g of dried plant/ 100mL), it was adjusted to *p*H 2.0 with HCl. 2mL porcine pepsin (3mg/mL) were added and it was incubated for 1 h at 37°C. The *p*H was adjusted to 5.6 with $NaHCO_3$ and 9mL of pancreatin (0.4mg/mL), pig bile extract (2.4mg/mL) and lipase (0.2mg/mL) was added. The *p*H was adjusted to 7.0 with NaOH and the mixture was incubated for 2 h at 37°C. The free metal content was assessed through ISE and the results shown in table 4 were calculated for 100mL infusion in light of all the dilutions made.

Experimental data showed that among the extracts that previously showed complexing capacity for metal cations, only infusions of *Vaccinium myrtillus* have retained that capacity under simulated digestive system conditions, and it was much reduced and only for $Cu^{+2}$, $Pb^{+2}$, and $Hg^{+2}$ cations. Extracts of *Rosa canina*, *Mentha piperita* and *Geranium robertianum* that were active previously, had lost their complexing ability or it was reduced to a level undetectable through the method used. One possible explanation is that changing the structure of the active principles involved in the complexation because of the enzyme mix, reduces the number of active centers available for attaching metal cations.

*Chemical composition of extractive aqueous solutions of Vaccinium myrtillus*

The active principles with complexing effect on metal cations under simulated digestive system conditions proved to be the anthocyanins. The anthocyanins are secondary plant metabolites, of flavonoids class, with high solubility and stability in aqueous medium. For the estimation of their concentration and that of their related compounds, the total polyphenols, total flavonoids and total anthocyanins contents were determined (table 5).

Total polyphenols content was determined using Folin-Ciocalteu method, by evaluating the -OH groups of the sample in alkaline medium ($Na_2CO_3$) [12]. The reagent, composed of a phosphotungstic acid and phosphomolybdic acid mixture, got reduced during the oxidation of phenols to a mixture of tungsten and molybdenum blue oxides and their absorbance measured at 765nm was proportional to the number of phenolic groups of anthocyanins. Total polyphenols content was expressed as gallic acid equivalents (GAE).

The total flavonoids content was determined using a colorimetric method [13]. 1mL sample was brought into a 10mL volumetric flask. 4mL double distilled water and 0.3mL 5% $NaNO_2$ were added and exactly five minutes later 0.3mL of 10% $AlCl_3$ followed. After one more minute had passed 2mL of 1M NaOH were added and the mixture was diluted with distilled water up to 10mL. The absorbance was measure at 510nm. The total content of flavonoids was expressed as quercetin equivalents (QE).

Total anthocyanins content was determined based on their property to change color depending on the *p*H [14]. Total anthocyanin content was expressed as cyanidin 3-galactoside equivalents (C3GE):mg of anthocyanins = (A x Mr) / ε where A = ($A_{510nm}$, *p*H = 1.0 - $A_{700nm}$, *p*H = 1.0) - ($A_{510nm}$, *p*H = 4.5 - $A_{700nm}$, *p*H = 4.5), Mr C3GE = 484.84 and the molar absorptivity of C3GE is ε = 34300.

## Conclusions

A mild detoxification that aims to reduce the bioavailability of heavy metals can be done resorting to natural solutions. Out of all the aqueous extracts tested, those derived from *Rosa canina*, *Mentha piperita*, *Geranium robertianum* and *Vaccinium myrtillus* proved to posses a measurable complexing capacity for the studied cations. Under simulated digestive system conditions, the complexing capacity was preserved only for *Vaccinium myrtillus,* in a much lesser extent, and only for $Cu^{+2}$, $Pb^{+2}$ and $Hg^{+2}$ cations. The high content of anthocyanins from *Vaccinium myrtillus*, compared to the other species studied, involve these active principles into the explanation on complexing metal cations. The study proved that oral administration of infusions of *Vaccinium myrtillus* has a moderate effect on reducing digestive bioavailability of heavy metals.

## References

1. CUCIUREANU, R., Elemente de igiena mediului si alimentului, Ed. Junimea, Iasi 2002, p. 44.
2. GOLDFRANK, L.R., FLOMENBAUM, N.E., LEWIN, N.A., HOWLAND, M.A., HOFFMAN, R.S., NELSON, L.S., Goldfrank's Toxicologic Emergencies, McGraw Hill, New York, 2002, p. 812.
3. ELLENHORN, M.J., Ellenhorn's Medical Toxicology, Williams & Wilkins, Philadelphia 1997, p. 1532.
4. KENNEDY, C.J., Encyclopedia of Fish Physiology, Academic Press, Amsterdam 2011, p. 2061.
5. SWARAN, J.S., FLORA, V.P., Int. J. Environ. Res. Public Health, **7**, 2010, p. 2745.
6. EBRAHIMZADEH, M.A., POURMORAH, F., BEKHRADNIA, A.R., Afr. J. Biotechnol., **7**, 2008, p. 3188.
7. ERDELYI POP, A., VAMANU, A., POP, O.V., VAMANU, E., Rev. Chim. (Bucharest), **66**, no. 10, 2015, p. 1687.
8. SOARE, L.C., VISOIU, E., BEJAN, C., DOBRESCU, C.M., FIERASCU, I., IOSUB, I., PAUNESCU, A., Rev. Chim. (Bucharest), **66**, no. 12, 2015, p. 2017.
9. SHIM, S.M., FERRUZZI, M.G., KIM, Y.C., JANLE, E.M., SANTERRE, C.R., Food Chem., **112**, 2009, p. 46.
10. AGOROAEI, L., BIBIRE, N., APOSTU, M., STRUGARU, M., GRIGORIU, I., BUTNARU, E., Rev. Chim. (Bucharest), **65**, no. 9, 2014, p. 1026.
11. APOSTU, M., BIBIRE, N., TANTARU, G., VIERIU, M., PANAINTE, A.D., AGOROAEI, L., Rev. Chim. (Bucharest), **66**, no. 5, 2015, p. 657.
12. WROLSTAD, R.E., ACREE, T.E., DECKER, E.A., Handbook of Food Chemistry, John Wiley & Sons, New Jersey, 2005, p. 37.
13. ZHISHEN, J., MENGCHENG, T., JIANMING, W., Food Chem., **64**, 1999, p. 555.
14. GIUSTI, M.M., WROLSTAD, R.E., Current protocols in food analytical chemistry, Wiley, New York, 2001, p. 121

Manuscript received: 18.12.2106