Total and extractable iron in selected herbs collected from natural areas in Northern Poland

PAWEŁ KONIECZYŃSKI, MAREK WESOŁOWSKI*, PIOTR RAFALSKI

Department of Analytical Chemistry
Medical University of Gdańsk
Al. Gen. J. Hallera 107
80-416 Gdańsk, Poland

*corresponding author: tel.: +4858 3493120, fax: +4858 3493124, e-mail: marwes@amg.gda.pl

S u m m a r y

The concentration of total iron and iron (II) extracted by hot twice distilled water was analyzed in following medicinal plants collected from natural areas: St Johns wort (*Hypericum perforatum* L.), yarrow (*Achillea millefolium* L.), nettle (*Urtica dioica* L.) and birch (*Betula pendula* Roth.). Total iron was determined by FAAS method after microwave mineralization of samples, whereas Fe (II) was extracted by twice distilled water in temperature of 85ºC and determined spectrophotometrically with use of o-phenantroline. The obtained results indicate that total iron content is from several dozen to more than 200 μg/g of dry plant weight and is correlated with plant species. In case of iron (II) it was determined in amounts ten times less than total concentration of the element. It was noticed that level of that form of iron is positively related to total amount of iron in analyzed medicinal plants. Extremely high concentration of both forms of iron was characteristic for one sample of nettle collected in the Vistula Sand Bar. Based on the conducted research it is possible to state that iron on +2 oxidation state extracted by water can become a source of bioavailable form of this element for human.

Key words: *total iron, iron (II), extraction, medicinal plants, collection from natural areas*

INTRODUCTION

Iron is one of the microelements indispensable for humans. Its delivery with food or herbal drugs can supply the organism with this essential metal [1]. The

analysis of plants used for medical purposes as a raw material comprises their chemical composition also in relation to their macro- and microelement contents. However, taking into consideration that medicinal plants are used as water extracts – herbal teas or infuses, it is important to recognize the bioavailable chemical forms of the investigated elements which are water soluble, and subsequently, absorbed in human digestive system. Therefore, the analysis of total concentration of essential elements (among them of iron) may not be sufficient to answer the question on the level of water extractable iron species, and how much it depends on total concentration of that element in a medicinal plant.

The speciation studies on the bioavailable forms of iron in medicinal plants used in China and extracts prepared from them have brought the information that the iron species detected in herbal drugs depended on the applied type of extraction solvent as well as on the analyzed botanical species [2, 3].

The deficiency of bioavailable form of iron on the +2 oxidation state causes many diseases. The most popular is anemia [4]. There was performed an investigation on the total and extractable iron species in eight African medicinal plants used traditionally against anemia. It was found that water extracts prepared from plants contained very low values of studied metal [5]. Moreover, the differences in total iron concentration in studied plant material were identified. They depended on the analyzed plant part as well as on the botanical species of a plant.

Among other elements, the total concentration of iron was often analyzed in medicinal plants and in infuses made from them. It was found that iron is poorly dissolved in water because only about 5–10% of total iron was determined in water extracts of medicinal plants [6-8]. The studies on water extractable species of iron in *herba hyperici* showed that it was determined in values from 3.4 to 11.9% of total iron in this plant [9].

Taking all of this into consideration, the aim of our investigation was to recognize the level of water extractable iron (II) in relation to total iron in four species commonly used in medicine collected from natural meadows in the Northern Poland in order to detect the differences between them as well as to identify the factors responsible for that differentiation.

MATERIALS AND METHODS

Plant material

Two collection areas were situated in Northern Poland in meadows in the suburbs of Gdańsk (Gdańsk Brzeźno and Gdańsk Oliwa – Dolina Radości), the third near small village Piaski on the Vistula Sand Bar, and the fourth in suburbs of Olsztyn-Jaroty. The medicinal plants were as follows: the whole plants of St Johns wort (*Hypericum perforatum* L. – samples 1, 2 and 3), yarrow (*Achillea millefolium* L. – samples 4, 5, 6 and 7), leaves of nettle (*Urtica dioica* L. – samples 8, 9, 10

and 11) and leaves of birch (*Betula pendula* Roth. – samples 12, 13, 14 and 15). Whole plants (or their leaves in case of nettle and birch) were cut, then dried in shadow in the temperature below 35˚C, ground using the Knifetec (Foss-Tecator, Denmark) sample mill and, subsequently, kept in polyethylene containers prior to the analysis.

Microwave digestion

To prepare the plant materials for total iron determination, accurately weighed samples (0.5 g) were digested with the mixture of 30% H_2O_2 (POChem, Poland) and concentrated 65% HNO_3 (Selectipur, Merck, Germany), (3:5, v/v) in Uniclever BM-1z (Plazmatronika, Poland) unit. Next the samples were transferred to 50 ml volumetric flasks and diluted with the twice distilled water obtained from the quarz-glass system (Heraeus, Switzerland).

Extraction

Twice distilled water of temperature of 85˚C was used for the extraction. To accurately weighed plant samples (about 1 g) 30 ml of hot water was added then stirred on electromagnetic stirrer for 30 min and filtered through paper filter with medium-sized pores (Filtrak, Germany). The filtrates were collected in the volumetric flasks and diluted to 50 ml with twice distilled water.

Determination

Concentration of total iron was determined with use of 250 Plus Atomic Absorption Spectrometer (Varian, Australia), applying standard conditions in air/acetylene flame at the analytical wavelength of 248.3 nm. The water extractable form of iron was determined basing on the reaction with o-phenantroline at analytical wavelength of 512 nm and cell path of 1 cm, using the UV/ViS spectrophotometer SP 870 (Metertek, South Korea). The recovery of methods applied for total iron checked with use of Virginia Tobacco Leaves 2 (CTA-VTL-2) certified reference material, and for Fe(II) determination obtained with use of standard addition method was equal to 92.1% and 95.0%, respectively [10].

Calculations

The correlation analysis was performed with the use of Statistica (Statsoft, Poland) software.

RESULTS AND DISCUSSION

As shown in Table 1, the results of the determination of total and extractable iron differed between analyzed plant samples. In general, the range of concentration in which total iron was found in all samples varied from 7.56 μg/g of dry weight (d. wt.) in one herb of St Johns wort (sample 1) collected in Gdańsk-Brzeźno, to 229.48 μg/g d. wt. in nettle (sample 10) harvested in Piaski. When comparing the average level of total iron in all analyzed samples, it is possible to notice that its concentration differentiated less among all four samples of birch (12-15) and four samples of yarrow (4-7) than in other two plant species. Total level of iron determined in all samples of nettle (8-11) and among analyzed samples of St Johns wort (1-3) was particularly different. These results indicate that total concentration of iron was determined on similar level in plant samples of the same botanical species. Therefore, such results confirm the conclusions of other researchers who also noticed that total iron content may depend on genetic factors. That means that several medicinal plants cumulate more iron in their tissues then others [6-9].

Table 1.

Results of determination of total and extractable iron in medicinal plants. The values are the range and the arithmetic mean of 6 determinations

sample number	collection area	total iron [μg/g d. wt.]	iron (II) [μg/g d. wt.]	iron (II)/total iron [%]
1	Gdańsk-Brzeźno Piaski Olsztyn-Jaroty	6.67–8.87; 7.56	6.19–6.22; 6.20	82.0
2		24.38–27.29; 26.06	3.99–4.05; 4,02	15.4
3		31.71–34.65; 32.91	6.37–6.46; 6.41	19.5
4	Gdańsk-Brzeźno	20.13–21.70; 20.79	3.24–3.31; 3.28	15.8
5	Gdańsk-Oliwa	24.62–28.46; 26.54	2.85–2.94; 2.89	10.9
6	Piaski	20.97–25.45; 23.26	4.78–4.82; 4.80	20.6
7	Olsztyn-Jaroty	16.13–16.55; 16.36	5.15–5.25; 5.20	31.8
8	Gdańsk-Brzeźno	58.93–74.83; 67.01	11.30–11.38; 11.34	16.9
9	Gdańsk-Oliwa	37.12–42.60; 40.17	11.26–11.31; 11.28	28.1
10	Piaski	223.82–235.37; 229.48	24.04–24.13; 24.09	10.5
11	Olsztyn-Jaroty	27.53–32.71; 30.76	11.35–11.43; 11.39	37.0
12	Gdańsk-Brzeźno	15.13–22.20; 18.41	1.63–1.69; 1.66	9.0
13	Gdańsk-Oliwa	26.91–27.11; 27.01	1.63–1.75; 1.70	6.3
14	Piaski	23.63–29.19; 26.48	0.70–0.79; 0.75	2.8
15	Olsztyn-Jaroty	17.72–18.53; 18.02	2.74–2.81; 2.77	15.4

Water extractable species of iron – Fe (II) (Table 1) differ less between samples of the same plant species than between different plants samples. The highest average level of iron (II) was found in all samples of nettle (samples 8-11), especially in the sample 10 harvested in Vistula Sand Bar. Lower amounts of Fe (II) were determined in all samples of St Johns wort (samples 1-3) and yarrow (samples 4-7), and the lowest in all samples of birch (samples 12-15).

In order to find a relationship between extractable iron and its total concentration in all analyzed plant samples the correlation analysis was performed (fig. 1).

The statistically significant ($\alpha<0.05$) linear correlation coefficient of 0.87, both calculated for all 15 samples, and the value of $r=0.61$ calculated for 14 samples (after discarding sample 10, due to its value outstanding from the other results) indicate a strong positive relation between water extractable iron and total iron in the set of investigated medicinal plants. Iron correlates positively with Al [11], Cu, Cr, Co and Mg [12] in plants, and negatively with Ca and Sr [12]. However, in this study the correlation between total iron and extractable iron was established for investigated plants collected in natural areas.

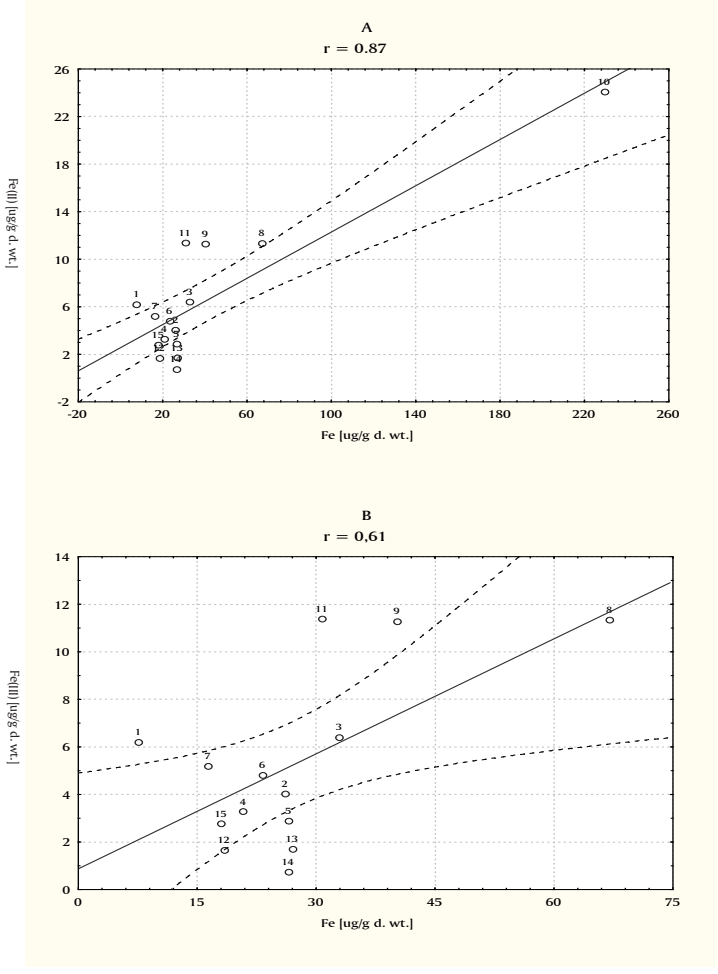

Figure 1.
Relation between total and extractable iron:
A) for 15 plant samples
B) for 14 plant samples (after elimination of sample 10 with extremely high concentration of iron)

Analysis of the extraction efficiency of twice distilled hot water used as extraction solvent is shown in the last column of Table 1. It is noticeable that Fe (II) level ranges from 2.8% of total iron in one of the birch samples (14) to 82.0% of total iron in the sample of St Johns wort (1). These two values are extremely low and high, respectively, but the average ratio of Fe (II)/total Fe is 18.2% of total iron in all plant samples, as calculated excluding the two extremes. This is an extraction ratio more similar to values known from the literature [6-9], indicating that preparation of water extracts of medicinal plants – infusions or decoctions may deliver to human digestive system rather low bioavailable fraction of total iron present in plant material.

CONCLUSIONS

The study on the total and water extractable iron species in four popular medicinal plants revealed that the amount of bioavailable iron is correlated to total concentration of that metal in all plant samples. Moreover, the preparation of hot water extracts, as commonly applied by people, can deliver 18% of the total iron present in a plant (on average) to the water extract, which can be treated as bioavailable fraction of iron. The differences in total and extractable iron level were more associated with the origin of the studied plants of different botanical species then to the fact that they were harvested in different areas.

REFERENCES

1. Kabata-Pendias A, Pendias H. Biogeochemia pierwiastków śladowych. 2nd ed., Warszawa 1999:329-36.
2. Li S, Deng N. Speciation analysis of iron in traditional Chinese medicine by flame atomic absorption spectrometry. J Pharm Biomed Anal 2003; 32:51-7.
3. Li S, Deng N, Zheng F. Effect of digestive site acidity and compability on the species, lipopily and bioavailability of iron, manganese and zinc in *Prunus persica Batsch* and *Carthamus tinctorus*. Bioorg Med Chem Lett 2004; 14:505-10.
4. Hołowiecki J. Choroby układu krwiotwórczego, in: Kokot F. Choroby wewnętrzne. 7th ed. Warszawa 2002:610-12.
5. Omolo OJ, Chhabra SC, Nyagah G. Determination of iron content in different parts of herbs used traditionally for anaemia treatment in East Africa. J Ethnopharm 1997; 58:97-102.
6. Szentmihalyi K, Then M. Teas of *Equiseti Herba, Myrtilli Folium* and *Salviae Folium*. Acta Aliment 2000; 29:43-9.
7. Łozak A, Sołtyk K, Ostapczuk P, Fijałek Z. Determination of selected trace elements in herbs and their infusions. Sci Total Environ 2002; 289:33-40.
8. Leśniewicz A, Jaworska K, Żyrnicki W. Macro- and micro-nutrients and their bioavailability in Polish herbal medicaments. Food Chem 2006; 99:670-9.
9. Zaręba S, Błoniarz J, Rahnama M. Studies on levels of iron and chromium in herbs and infusions obtained from them used by adults and children. Bromat Chem Toksykol 2003; 36:317-25.
10. Konieczyński P, Wesołowski M. Water extractable forms of nitrogen, phosphorus and iron in fruits and seeds of medicinal plants. Acta Pol Pharm Drug Res 2007; 64: 385-91.
11. Razic S, Onjia A, Dogo S, Slavkovic L, Popovic A. Determination of metal content in some herbal drugs – Empirical and chemometric approach. Talanta 2005; 67:233-9.
12. Rashed MN. Trace elements in some wild plants from the shores of the high dam lake and the adjacent desert, as determined by atomic absorption spectroscopy. J Arid Environ 1995: 29:185-97.

ŻELAZO CAŁKOWITE I ULEGAJĄCE EKSTRAKCJI W ZIOŁACH ZEBRANYCH ZE STANU NATURALNEGO W POLSCE PÓŁNOCNEJ

PAWEŁ KONIECZYŃSKI, MAREK WESOŁOWSKI*, PIOTR RAFALSKI

Katedra i Zakład Chemii Analitycznej
Akademia Medyczna
Al. Gen. J. Hallera 107
80-416 Gdańsk

*autor, do którego należy kierować korespondencję: tel.: +4858 3493120, faks: +4858 3493124, e-mail: marwes@amg.gda.pl

S t r e s z c z e n i e

Analizie poddano zawartość żelaza całkowitego oraz żelaza (II) ulegającego ekstrakcji gorącą wodą redestylowaną w następujących roślinach leczniczych zebranych ze stanu naturalnego: dziurawcu zwyczajnym (*Hypericum perforatum* L.), krwawniku pospolitym (*Achillea millefolium* L.), pokrzywie zwyczajnej (*Urtica dioica* L.) oraz brzozie brodawkowatej (*Betula pendula* Roth.). Żelazo całkowite oznaczono metodą FAAS po uprzedniej mineralizacji mikrofalowej próbek, natomiast Fe (II) ekstrahowano wodą redestylowaną o temp. 85ºC i oznaczano spektrofotometrycznie, stosując o-fenantrolinę. Uzyskane wyniki wskazują, że zawartość żelaza całkowitego stanowi od kilkudziesięciu do ponad 200 μg/g suchej masy surowca, i jest uzależniona od przynależności gatunkowej analizowanej rośliny. W przypadku żelaza (II), oznaczono go w ilości dziesięciokrotnie niższej niż całkowitą zawartość pierwiastka. Stwierdzono, że poziom tej formy żelaza wykazuje korelację dodatnią z zawartością żelaza całkowitego w badanych roślinach leczniczych. Szczególnie wysoką zawartością obu form żelaza charakteryzowała się jedna z próbek pokrzywy zebrana na Mierzeji Wiślanej. Na podstawie przeprowadzonych badań można stwierdzić, że żelazo na +2 stopniu utlenienia, ulegające ekstrakcji wodą, może stanowić źródło biodostępnej formy tego pierwiastka dla organizmu człowieka.

Słowa kluczowe: *żelazo całkowite, żelazo(II), ekstrakcja, rośliny lecznicze, zbiór ze stanu naturalnego*