ORIGINAL ARTICLE

VARIATION OF IRON CONTENTS, POLYPHENOLS AND FLAVONOIDS IN *PETROSELINUM CRISPUM* (MILL.) FUSS (*APIACEAE*)

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Abstract

Petroselinum crispum (Mill.) Fuss., parsley, *Apiaceae*, is often used in the folk therapy as an anti-anaemic product and is claimed to be rich in iron. Previous reports have stated iron contents in leaves varying between 126.2 and 1100 mg/kg, but no report on plants cultivated in Romania are available. We have measured iron contents in the vegetative organs (roots, stems and leaves) of plants cultivated in Romania on three different soils, at three development stages, using AAS. Since iron absorption may be influenced by the polyphenols and flavones, we have also measured these phytochemicals spectrophotometrically, using the Folin-Ciocâlteu and aluminium chloride chelation methods, respectively. Correlations with the soil chemical composition were also explored. The iron contents in all vegetative organs of the plant varied between 21.6 (s.d. 10.8) in stems and 645.2 (21.9) mg/kg in leaves. The iron contents tended to increase with the development stage and the soil type had very limited influence on the iron contents. Polyphenol and flavone contents varied by soil type, vegetative organ and development stage, with interactions among these variables.

Rezumat

Petroselinum crispum (Mill.) Fuss., pătrunjel, fam. *Apiaceae*, este adesea utilizat în terapia populară ca produs antianemic și se pretinde că este bogat în fier. Rapoarte anterioare au afirmat un conținut în fier variind între 126.2 și 1100 mg/kg, dar nu este disponibil nici un raport asupra plantelor cultivate în România. S-a măsurat conținutul de fier în organele vegetative (rădăcini, tulpini și frunze) ale plantelor cultivate în România pe trei soluri, la trei stadii de dezvoltare, utilizând SAA. Deoarece absorbția fierului poate fi influențată de polifenoli și flavone, am măsurat și acești compuși fitochimici, spectrofotometric, utilizând metodele Folin-Ciocâlteu și, respectiv, pe baza chelatării cu clorură de aluminiu. Au fost explorate și corelațiile cu compoziția chimică a solului. Conținutul în fier al tuturor organelor vegetative ale plantelor a variat, între 21.6 (d.s. 10.8) în tulpină și 645.2 (21.9) mg/kg în frunze). Conținutul de fier a tins să crească odată cu stadiul de dezvoltare, iar tipul de sol a avut o influență foarte limitată asupra conținutului de fier. Conținutul de polifenoli și flavone a variat în funcție de tipul de sol, organul vegetativ și stadiul de dezvoltare, cu interacțiuni între aceste variabile.

Keywords: *Petroselinum crispum*, polyphenols, flavonoids, iron

Introduction

Petroselinum crispum (Mill.) Fuss, parsley, fam. *Apiaceae*, is a biennial herb used for centuries for its aromatic properties as a spice and vegetable (mostly the leaves, but the subterraneous parts are also consumed), and is nowadays cultivated worldwide, being employed both as a food and as a raw material in the cosmetics industry [3, 11]. The leaves contain essential oil having as major compounds myristicin, a phenylpropene derivative named apiol(e)) (but an extract obtained from the plant is also known as apiol), α-pinene, β-pinene, 1-allyl-2,3,4,5-tetramethoxy-benzene, 1,3,8-p-menthatriene, β-phellandrene and myrcene, trans-β-ocimene, γ-terpinene, 1-methyl-4-isopropenyl benzene, terpineolen(e), β-elemene, and many minor additional compounds [19, 20, 21, 35, 41]. The aerial parts of the species also contain monoterpene derivatives (petroside) [38], flavonoids (apigenin, apigenin-7-O-glucoside or cosmosiin or apigetrin, apigenin-7-O-apiosyl-(1-> 2)-O-glucoside or apiin, 6″-0-acetylapiin, malonyl apiosylglucoside, diosmetin 7-O-β-D-glucopyranoside, kaempferol 3-O-β-D-glucopyranoside) [8, 15, 38] coumarins (mostly furocoumarins: cnidilin, isoimperatorin, 2",3"-di-hydroxyfuranocoumarin or oxypeucedanin hydrate, psoralen, 5-MOP, 8-MOP, isopimpinellin) [8, 7, 38] (benzyl acuminose, also known as icariside F2) [38], carotenoids (β-carotene, lutein, violaxanthin, neoxanthin) [14], and ascorbic acid [9].

Extracts or the essential oils obtained from the root or aerial parts of *P. crispum* have been investigated in animal or cellular experimental settings for a variety of putative benefits and pharmacological activities: protection against hepatic and cardiac toxicity of cisplatin [1], against cadmium-induced toxicity [5, 22] and against hepatic steatosis [23]; as a potential remedy for male infertility [30] and diabetes [2]; for immunosuppressive effects of potential use in different conditions [39]; for antiproliferative effects in breast cancer cells[32]; for antibacterial, antifungal [20, 34, 35], antimosquito activities [17] and others.

According to popular wisdom in Romania, the plant is rich in iron and is recommended as a folk remedy against iron deficiency anaemia, oftentimes with claims that it is rich in iron. A few scientific publications have reported iron contents in parsley, with amounts varying between 126.2 and 543.5 [4], 81.26 and 605.50 [10], and even 1100 mg/kg [29] in leaves, and 406 mg/kg in fruits [24]. We were not able to find any data on iron contents in the roots or stems of the species. We have also not found reports on iron contents in plants cultivated in Romania, and this study reports on iron contents in vegetative organs of the species cultivated in Romania. Because iron absorption is often influenced by the polyphenols and flavonoids, we have also looked at the variation of these phytochemicals in different parts at three stages of development of the plant.

Materials and Methods

Herbal material

The herbal material was furnished by S.C. Hofigal S.A. and S.C. AgroecoBioterra S.R.L. (Bucharest) from their own cultures obtained in ecological conditions. Voucher specimens (no. 24-50/2015) are kept at the Pharmaceutical Botany and Cell Biology department. The identity of the species was confirmed by macroscopic and microscopic analysis. The plants were cultivated on three distinct sites, one in Transylvania (a preluvosol with clay texture, rich in carbonates derived from carbonate rocks – hereafter abbreviated as P) and two in the South of Romania (a preluvosol type soil - a brown-reddish forest soil, with a clay loam, sandy texture and glomerular structure, hereafter abbreviated as B soil, and a chernozem soil of argic and cambic type, rich in humus – hereafter, C soil). The plants were harvested at three stages of ontological development: before flowering (stage I), at the start of flowering (flower buds emergence – stage II) and at anthesis (stage III), separated in the three main vegetative organs: root, stem and leaves and dried. All results are reported on a dry weight basis.

Assay of iron and other minerals

The contents in iron and other oligoelements were measured using atomic absorption spectrometry (AAS), according to the European Pharmacopoeia, 9th edition [42].

Soil analysis

The soil contents in macro- and microminerals were assessed through atomic absorption spectrometry (AAS) using the U.S. EPA (1992) M-7000A [36], Test Method and SR ISO 11047:1999 [18].

Total polyphenol assay

The total amount of polyphenols was assayed with the Folin-Ciocâlteu spectrophotometric method [16, 33]. The pulverized herbal material (< 250 μm), in samples of 100-220 mg was extracted with methanol 70% in a volumetric flask of 20 mL (for roots and stems) or 50 mL (for leaves), using an ultrasound bath (Elmasonic S15H), for 15 minutes at 25°C. An aliquot of 10 mL was taken and subject to centrifugation for 10 minutes and 6000 rpm (Centurion Scientific C2 centrifuge), and a further aliquot of 1 mL was brought from the supernatant in a 10 mL volumetric flask. 5 mL of Folin-Ciocâlteu reagent diluted in water (1:10) were added to the solution and the flask was filled to the mark with a Na_2CO_3 7.5%. A control sample was prepared in the same conditions, where the Folin-Ciocâlteu reagent was substituted by distilled water. The prepared samples were kept protected from light for 1 hour and the absorbance was measured at 765 nm using a HALO DB-20 UV-VIS spectrophotometer (Dynamica Ltd., Austria). Gallic acid in methanol 70% was used to build a 7-point calibration scale (r = 0.9996).

Flavonoid assay

The total amount of flavonoids was assayed with the spectrophotometric method based on chelation with $AlCl_3$ in sodium acetate (Romanian Pharmacopoeia, 10th edition, *Cynarae folium* monograph), processing the herbal material in a similar way with that for polyphenols and measuring absorbance at 430 nm, against a 10-point calibration scale with quercetin (r = 0.9998).

Statistical analysis.

All statistical analyses were carried out in the R computing environment, version 3.4.1. [28]. The complex relationship between herbal part, type of soil and development stage for iron contents were conducted using generalized least squares (gls) with serial "corARMA" correlation structures (as the Durbin Watson test indicated the presence of autocorrelated errors in multiple linear models explored), using the R package nlme [27]. The relationship between the main factors analysed and the polyphenol contents was modelled using weighted multiple regression ("car" R package [12] and "sandwich" [40]), as the data were heteroscedastic. Effects computation and plotting was carried out with the "effects" R package

[13]. Model validity assumptions were checked using graphical means (leverage plots, quantile-quantile plots, histograms of studentized residuals), as well as inferential tests (taking into account the limitations of each such tool), with the help of the "car" [12], "MASS" [37] and "gvlma" [25] packages. We explored the correlations among polyphenols, flavones and iron concentrations with Pearson (assuming a linear relationship) and Spearman (assuming a monotone relationship) correlation tests.

Results and Discussion

The variation of iron contents in the three herbal parts by stage of ontological development and by soil (cultivation location) are shown in Fig. 1, A-C, whereas the mineral contents of the three soils (3, 5 and 4 samples, respectively,) is included in Table I.

Table I

Chemical composition of the soils on which the plant analysed was cultivated

Soil	Fe	Na	K	Ca	Mg	Cd	Cu	Cr	Mn	Zn
B	3.745	561.97	9599.91	11477.12	7390.03	0.45	240.09	58.17	988.98	289.42
B	3.358	608.84	18181.17	15539.26	7973.78	0.38	218.7	52.74	873.75	206.55
B	3.833	589.55	22981.09	12346.7	8686.75	0.34	209.83	65.29	1033.4	177.74
P	4.62	612.19	12837.1	7650.05	842.24	< 0.25	27.86	76.03	952.93	75.46
P	4.363	621.96	13570.05	6790.05	8041.51	< 0.25	30.16	78	1034.84	72.88
P	3.954	632.4	12822.27	6886.68	7917.44	< 0.25	29.88	75.99	1054.16	73.7
P	4.552	611.33	13309.34	7778.07	8865.38	< 0.25	29.28	74.89	984.44	78.5
P	4.129	583.48	12896.8	6732.32	7603.34	< 0.25	31.42	72.86	1038.96	73.15
C	4.112	587.19	11985.61	7961.09	8598.85	< 0.25	27.67	70.29	1010.75	69.05
C	4.218	600.71	12136.22	7784.76	9792	< 0.25	27.98	75.21	965.53	70.81
C	4.144	584.87	9060.01	7301.74	8815.81	< 0.25	28.43	73.65	1061.32	71.8
C	4.389	563.29	11635.76	7843.1	9356.34	< 0.25	28.02	72.37	1028.24	73.04

In partial agreement with the widespread folk claims of parsley being a herb rich in iron, with the high potential of use in treating anaemia, in the plants analysed by us from different cultures and at different development stages, the iron amounts accumulated by this popular aromatic herb were relatively high in root and leaf but low in stem, varying between 408.5 (s.d. 21.5) and 645.2 (21.9) mg/kg in leaves, 21.6 (s.d. 10.8) and 130.6 (21.8) mg/kg in stems, and 324.7 (s.d. 21.7) and 612.9 (21.5) mg/kg in roots. This is higher than what has been reported in the literature for many species, but not excessively so (in the literature the median value in leaves tends to be higher than 150 mg/kg, but in certain species iron levels may be well over 1000 mg/kg) [6].

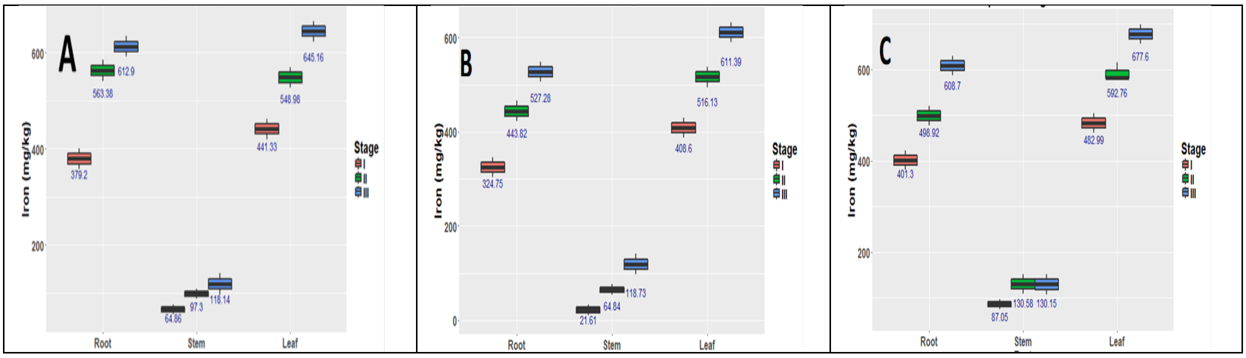

Figure 1 (A - C).

Boxplot-and-whiskers graph showing the variation of iron concentration by organ, development stage and soil. The numbers under the boxplots indicate the mean (n = 3)

Similarly to our previous findings in *Mentha x piperita* L., the iron concentration tended to increase with the development stage ($p < 0.001$, generalized least squares regression with second degree autoregressive errors), a feature that has also been reported in other species, such as *Prunus avium* L., *Phaseolus vulgaris* L. [31], but not in all [26]. There were also significant differences ($p < 0.001$) in content between root, stem and leaves, with the highest in leaves, close to the content in roots, and considerably lower contents in stem. There were no significant differences between soil B and C (the latter with a slightly, but insignificantly higher contents, $p = 0.30$), but plants grown on soil P had significantly lower iron levels than soil B ($p < 0.001$). However, the difference in effects was relatively small (effect size was 387.1 for soil B, 399.5 for soil C, and 337.7 for soil P, with a range for the effect of only 61.86, which means that the difference of concentration from one soil to another is less than 62 mg/kg, when all other variables are kept constant – Figure 2).

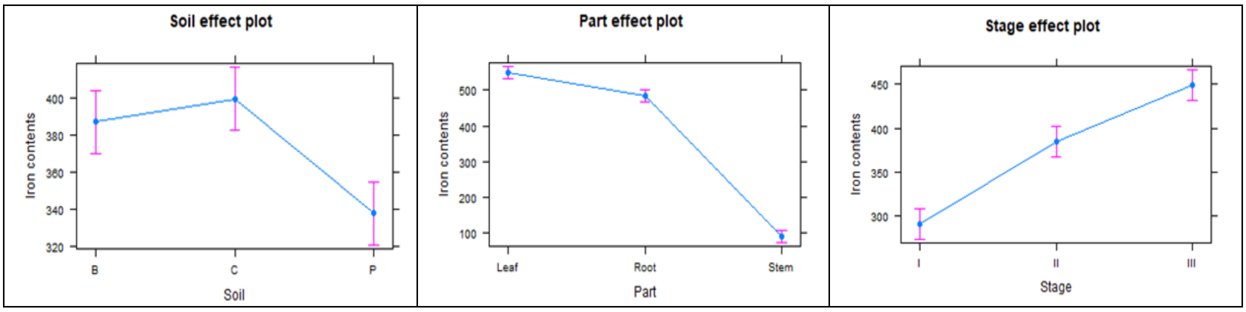

Figure 2.
Effects plots for the three variables influencing iron contents in *Petroselinum crispum* (Mill.) Fuss

We have examined the potential correlation between the soil mineral composition and the iron contents in the different organs of the species cultivated on different soils, taking into account the limited number of data points available (which requires caution in interpreting the findings). The iron contents in soil was correlated rather negatively with the iron amounts in the plant, particularly in roots (for leaf, r = -0.10 (Pearson, apt at measuring linear relationships) and r = -0.5 (Spearman, apt at detecting monotonic relationships, e.g. sigmoid); for stem, Pearson r = -0.18 and Spearman r = -0.5; for root, Pearson r = -0.74 and Spearman r = -1. Na^+ levels in soil were also strongly and negatively correlated with the iron contents in *Petroselinum crispum* (Pearson r < -0.88 for each of leaf, stem and root), whereas soil Mg^{2+} was positively correlated with the iron content in the plant (r = 0.80 for root, r > 0.99 for leaf and stem).

There were multiple statistically significant interactions ($p < 0.001$) among soil type, herbal part and development stage and polyphenol concentration. The sense of the interactions and the effects of soil and development stage for each organ is shown graphically in Figures 3, 4 and 5. Although we have measured three factors with potentially high impact on iron and phytochemical composition, each soil types actually comes with additional factors that may confound results, and thus the differences found and reported here my not reflect only soil contribution, but also other external variables, such as temperature and humidity variations, pests to which the plants were exposed etc.

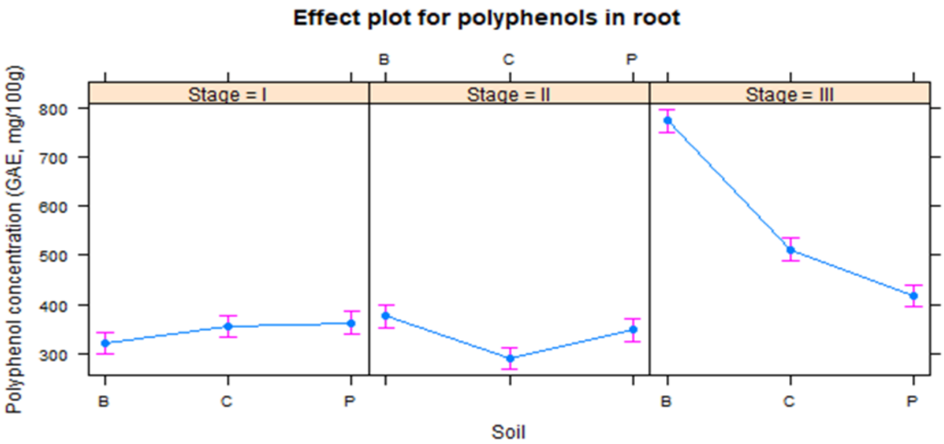

Figure 3.
Graphical representation of the interactions between development stage, soil type and polyphenols in the roots of *P. crispum* (Mill.) Fuss

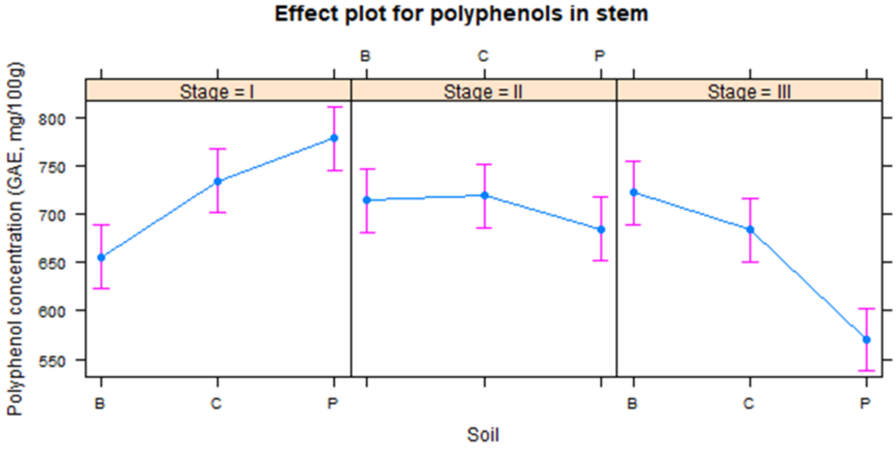

Figure 4.
Graphical representation of the interactions between development stage, soil type and polyphenols in the stems of *P. crispum* (Mill.) Fuss

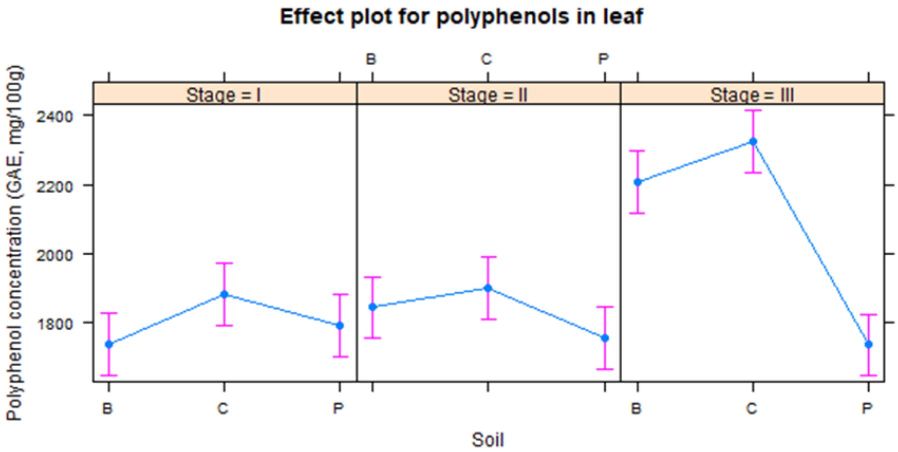

Figure 5.
Graphical representation of the interactions between development stage, soil type and polyphenols in the leaves of *P. crispum* (Mill.) Fuss

The variation of flavone contents in the three organs of the species by development stage and type of soil is shown in Table II. The lowest concentrations were found in root, close to those in stem, and considerably lower than the contents found in leaf. Surprisingly, for the herbal product collected in stage I of development the flavone content was under the limit of quantification, which may be due to the low level of biosynthesis in the first part of the plant existence. The largest interactions were seen for the leaf concentrations: in the plants cultivated on preluvosol type soils they tended to decrease between stages II and III, whereas in the plants grown on chernozem it had a contrary trend.

Table I
Variation of flavone contents (mean, mg/100 g) (s.d.) in organs of *P. crispum* (Mill.) Fuss. by development stage and soil type

Soil type	***Development stage***		
	Root		
	I	**II**	**III**
B	NA*	61.67 (2.07)	57.50 (2.08)
P	NA*	42.80 (1.97)	33.78 (1.52)
C	NA*	41.79 (1.61)	38.54 (1.57)
	Stem		
	I	**II**	**III**
B	NA*	78.23 (2.13)	50.06 (1.74)
P	NA*	69.02 (3.24)	153.47 (4.77)
C	NA*	107.29 (2.87)	108.25 (4.04)
	Leaf		
	I	**II**	**III**
B	NA*	345.11 (14.39)	172.61 (5.16)
P	NA*	296.26 (14.62)	148.77 (6.63)
C	NA*	228.50 (7.14)	442.22 (20.86)

NA – not available (as explained in the text, the content was under the limit of quantification)

Conclusions

Somewhat in agreement with the folk traditions, the iron contents of *Petroselinum crispum* (Mill.) Fuss was higher than the median values reported in the literature for other herbal species. It would also not be impossible for the plant to act through other mechanisms in the sense of improving anaemia (e.g. by interference with hepcidin signalling), but alternative mechanisms of anti-anaemic activity were not investigated in our study. The iron levels tended to increase with the development stage, whereas polyphenols and flavone contents varied more broadly, with important interactions among type of soil, herbal part and development stage.

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