ORIGINAL ARTICLE

VARIATION OF POLYPHENOLS AND IRON CONCENTRATION IN *MENTHA X PIPERITA* L. BY DEVELOPMENT STAGE AND SOIL TYPE

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Abstract

The variation of polyphenols, flavonoids and iron concentrations in the different organs of *Mentha x piperita* L. by soil type and ontogenetic development stage of the plant is less known. We have investigated the quantitative variation of total polyphenols (spectrophotometrically, using the Folin-Ciocâlteu method), total flavonoids (spectrophotometrically, with the aluminium chloride chelation method) and iron in roots, stems and leaves of this species at three development stages, cultivated on three different soils (a brown-reddish forest soil, chernozem soil of argic and cambic type, rich in humus, and a preluvosol with clay texture, rich in carbonates derived from carbonate rocks). Significant interactions were found between soil and development stage when assessing the relationship between total polyphenols, flavonoids, iron and these two nominal variables. The highest level of total polyphenols was found in roots and subterraneous organs, while leaves were richest in flavonoids (not quantifiable in stems and subterraneous organs). The iron concentration tended to increase with each development stage in all three organs. There is virtually no correlation between total polyphenols or flavonoids and iron, but a monotonic relationship was confirmed between polyphenol and flavonoid levels.

Rezumat

Variația concentrației polifenolilor, a flavonoidelor și a fierului în diferitele organe ale speciei *Mentha x piperita* L. sub influența diferitelor tipuri de sol și a stadiului de dezvoltare ontogenetică este puțin cunoscută. Am investigat variația cantitativă a polifenolilor totali (spectrofotometric, utilizând metoda Folin-Ciocâlteu), a flavonoidelor totale (spectrofotometric, prin metoda chelatării cu clorură de aluminiu) și a fierului în rădăcinile, tulpinile și frunzele acestei specii în trei stadii de dezvoltare, din specimene cultivate în trei soluri diferite (un sol brun-roșcat de pădure, un cernoziom de tip argic și cambic, bogat în humus și un preluvosol cu textură argiloasă, bogată în carbonați). Au fost observate interacțiuni semnificative între tipul de sol și stadiul de dezvoltare la evaluarea relației dintre polifenolii totali, flavonoidele totale, fier și aceste două variabile nominale. Nivelul cel mai ridicat de polifenoli totali a fost observat în rădăcini și organe subterane, în timp ce frunzele au fost cele mai bogate în flavonoide (acestea nu au fost cuantificabile în tulpini și organe subterane). Concentrația de fier a crescut odată cu stadiul de dezvoltare în toate cele trei organe. Nu există practic nici o corelație între polifenolii totali sau flavonoidele totale și fier, dar o relație monotonă a fost confirmată pentru conținutul în polifenoli și flavonoidelor.

Keywords: *Mentha x piperita* L., polyphenols, flavonoids, iron, soil types, development stage

Introduction

Mentha x *piperita* L. (peppermint), *Lamiaceae*, is a hybrid of *M. spicata* L. (spearmint) and water mint (*M. aquatica* L.), indigenous in Europe (where is also widely cultivated), naturalized on the American continent and cultivated in other regions of the world for its essential oil and fragrance properties, widely applied in the pharmaceutical, cosmetic and food fields [26]. Antiallergenic, antiviral, antibacterial and cytotoxic activities have been attributed to the whole aerial parts of the species [26, 41]. In the traditional medicine of various peoples it is extensively used for its putative anti-spasmodic, carminative, anthelmintic or anti-motion sickness effects [42], as well as in the treatment of nausea and vomiting, stimulation of appetite, fever, colds or inflammations [42]. The essential oil tends to be more widely explored for a variety of indications: as an anti-helminthic [35], antibacterial [23], insect repellent [17], in the treatment of spasms, colds, indigestions, nausea, sore throat, toothaches [23], removing malodour and volatile sulphur compounds [18] and even cancer [23]. Despite the long tradition of medicinal use for this species, the small number of clinical trials assessing its efficacy or safety is rather surprising.

Besides essential oil (0.5 - 4.0 %) [39], the herb (mostly in leaves) contains caffeic acid, rosmarinic acid [9] and flavonoids: eriodictyol-7-rutinoside (eriocitrin, seemingly the majoritary compound, representing about 80% of the total flavonoids in one study [9], but only 38% in another [39]) [16, 20], luteolin [14], luteolin-7-O-rutinoside [16, 20], naringenin-7-O-glucoside [9], hesperetin 7-rutinoside (hesperidin), naringenin-7-O-rutinoside (narirutin), apigenin-7-O-rutinoside (isorhoifolin), diosmetin 7-O-rutinoside (diosmin), 5,7-dihydroxycromone-7-O-rutinoside [20]. We could not find data on the variation of the polyphenol or flavonoid content at different life stages of the plant or the potential influence of soil on the content in these compounds. As all plants, *M. piperita* contains small amounts of iron and other minerals. Its leaf iron content reported in the literature varied greatly, from 29.2 to 1154 mg/kg [8, 24, 27, 31, 33, 34, 36, 40]. We could not find any data on its iron contents in root or stem. It has been reported that peppermint tea (as other herbal teas rich in polyphenols), is able to inhibit considerably the absorption of iron from meals [19], which suggest that leaves containing moderate amounts of iron may have a rather negative effect on iron absorption in humans. It seems reasonable to assume that total polyphenols and flavonoids, as well as other minerals are influenced by the development stage and the type of soil a plant is grown in. In this context we report on our assessment of the influence of the growth stage and soil on flavonoid and polyphenol content in *Mentha x piperita* L., as well as on iron and on potential correlations between these nutrients.

Materials and Methods

Herbal material

The herbal material was provided by S.C. Hofigal S.A. and S.C. AgroecoBioterra S.R.L. (Bucharest, Romania) from ecological cultures. The three main vegetative organs (root, stem and leaf) were collected at three stages of plant development: before blooming (stage I), at blooming initiation (flower buds emergence – stage II) and full bloom (anthesis – stage III). The plants were cultivated in three different soils, two in the South of Romania (a brown-reddish forest soil with clay loam, sandy texture and glomerular structure – hereafter, B soil - and a chernozem soil of argic and cambic type, rich in humus – hereafter, C soil) and one in Transylvania, Romania, (a preluvosol with clay texture, rich in carbonates derived from carbonate rocks – hereafter, P soil).

Total polyphenol assay

The total phenolic content was estimated based on the Folin-Ciocâlteu spectrophotometric method (gallic acid equivalence method) [22, 38]. The herbal material was powdered using an electric mill and sieved by a sieve with a mesh size of 250 μm. Amounts varying between 60 and 240 mg were accurately weighed and extracted with 70% methanol in a volumetric flask of 20 mL (for roots and stems) or 50 mL (for leaves), at 25°C, in an ultrasound bath (Elmasonic S15H), for 15 minutes. An aliquote of 10 mL was centrifuged for 10 minutes and 6000 rpm (Centurion Scientific C2 centrifuge). An aliquot of 0.5 or 1 mL was transferred from the supernatant in a 10 mL volumetric flask. To this, 5 mL of Folin-Ciocâlteu reagent (diluted 1:10 with water) were added, and an aqueous solution of 7.5% Na_2CaO_3 was used to fill the flask to the mark. In parallel a control sample was prepared in a similar way, but the Folin-Ciocâlteu reagent was replaced by distilled water. The flasks were kept in dark for 1 hour at room temperature, after which the absorbance was read at 765 nm (a HALO DB-20 UV-VIS spectrophotometer, Dynamica Ltd., Austria). A 7-point calibration scale was prepared using gallic acid in 70% methanol, for a range of concentrations varying between 1.31 and 11.79 mg/L (r = 0.9996).

Flavonoid assay

The total flavonoid content was estimated using the spectrophotometric method with $AlCl_3$ in sodium acetate (Romanian Pharmacopoeia, 10th edition, *Cynarae folium* monograph [1]). The herbal material was powdered and sieved in the same way as for polyphenols. Amounts varying between 140 and 220 mg were accurately weighed and extracted with 70% methanol in a volumetric flask of 20 mL, at 25°C, in an ultrasound bath (Elmasonic S15H), for 15 minutes. An aliquot of 10 mL was centrifuged for 10 minutes and 6000 rpm. An aliquot of 2 mL was transferred from the supernatant in a volumetric flask of 10 mL, and 2 mL of 10% sodium acetate and 1 mL of 0.25% $AlCl_3$ were added, making up to 10 mL with 70% methanol. After a waiting period of 15 minutes the absorbance was measured at 430 nm. A 10-point calibration scale was prepared using quercetin in 70% methanol, for a range of concentrations varying between 0.8 and 20 mg/L (r = 0.9998).

Assay of iron and other minerals

The contents in iron and other oligo elements (zinc, chromium, lead, copper, manganese and cadmium) were measured using atomic absorption spectrometry (AAS), according to the European Pharmacopoeia, 9th edition [2]. 200 - 500 mg of powdered material was weighed in a crucible and calcined at 600 - 800°C for four hours. After calcination and cooling off 5 mL of a mixture of HCl:water 1:1 (v/v) were added and then evaporated to dryness. The ash residue was quantitatively transferred from the crucible in a 50 mL volumetric flask by three consecutive washings with 2.5 mL of a HCl:water mixture (1:5, v/v). 5 mL of a HNO_3:water mixture (v/v) were added in the crucible and again evaporated

to dryness. The residue was also transferred by repeated washings with 2.5 mL of a HCl:water mixture (1:5, v/v), the fractions being collected in the same volumetric flask. The crucible was then washed with water until the flask was filled up to the mark. The blank consisted of a 1% HNO_3 solution free of heavy metals. The absorbance of reference and test solutions was measured against the blank. The wavelength for each mineral element was: Pb = 217.0 nm; Fe = 248.3 nm; Zn = 213.9 nm; Mn = 279.5 nm; Cr = 270.1 nm; Cu = 324.8 nm; Cd = 228.8 nm. An acetylene-air flame was used, with a rate of 1.6 L/min. for acetylene and a lead lamp.

Statistical analysis

All statistical analyses were performed using the R computing environment, version 3.3.2 [32]. Modelling the relationship between organ, soil and development stage for polyphenols and iron and between soil and development stage for flavonoids was performed separately for each organ and each soil type using mostly generalized least squares (GLS) with an auto-regressive moving average process, with arbitrary orders for the autoregressive and moving average components (corARMA) (*nlme* R [30]) and where appropriate, multiple linear regression, because our attempt at modelling simultaneously the data by the three variables with GLS found numerous interactions between the three factors. The validity assumptions were verified graphically (leverage plots, quantile-quantile plots, histograms of studentized residuals) and by various inferential tests, using the "car" [12], "MASS" [43] and "gvlma" [28] packages. Correlations between iron, total polyphenols and flavonoid concentrations were evaluated using Pearson and Spearman tests (assessing linear and monotone relationships, respectively); the statistical significance of the correlation coefficients were computed using the Hmisc R package [15].

Results and Discussion

The variation of total polyphenol contents by soil and development stage is shown in Figure 1. For all three organs, significant interactions ($p < 0.05$, most often $p < 0.001$) between soil and stage were detected by the GSL models applied, the strongest effect being generally seen for soil C, where for all three organs the time evolution of polyphenol concentration tended to be rather U-shaped. For soils B and C, the polyphenol concentration tended to be maximal in the first stage, whereas for soil P it tended to be maximal in the third stage. Polyphenol concentration tended to be maximum in subterraneous organs, minimal in stems and to have intermediary levels in leaves.

The total amount of polyphenols (flavonoidic and non-flavonoidic) in mint leaves has been estimated to be up to 21.7% [39].

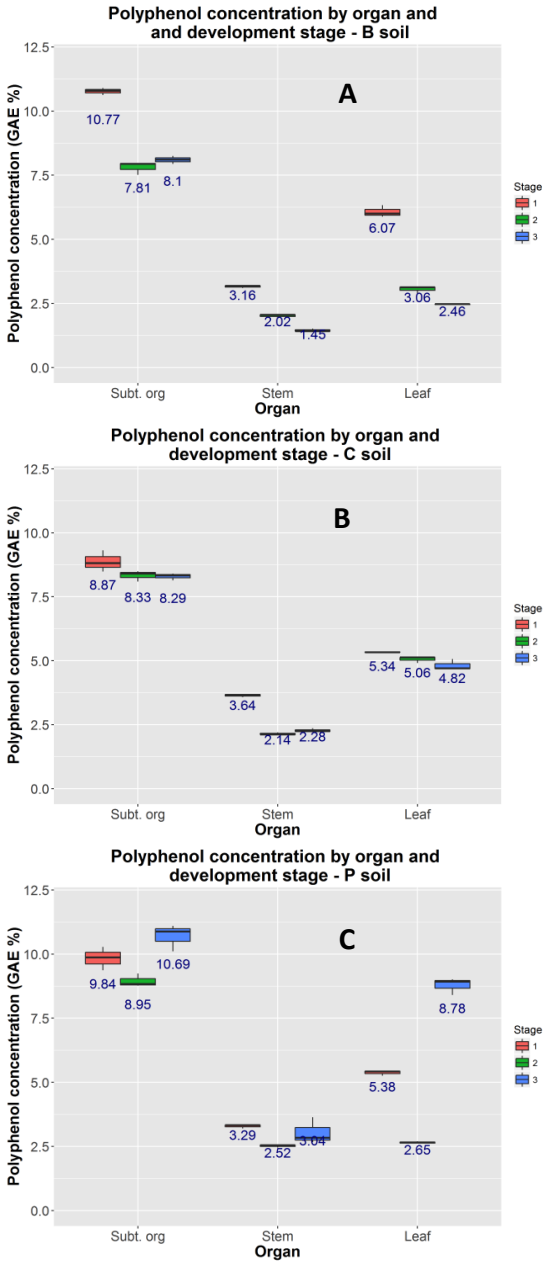

Figure 1.
Boxplot-and-whiskers graph showing the variation of total polyphenols by organ, development stage and soil. The numbers under the boxplots indicate the mean (n = 3).

A total polyphenol content of 4.11 - 4.32% (gallic acid equivalents, GAEs) has been reported in samples of commercial origin from the Romanian market, based on the spectrophotometric method with sodium nitrite and sodium molybdate in an alkaline medium [4]. Using a different analytical method, we obtained somewhat comparable results across different samples, depending on soil and development stage, results close to the same order of magnitude as the ones reported in the national literature, with variations between 2.46 and 8.78 % (GAEs). Our report seems

to be the first assessing the total polyphenol levels in roots and stem of this well-known hybrid, and it is interesting that the levels of total phenolics tend to be higher in the subterraneous organs (range 7.81 - 10.77% GAEs) rather than in leaves, whereas in stems they are at the lowest level (range 1.45 - 3.64% GAEs) among the vegetative organs.

Table I

Variation of flavonoid contents in leaf by stage and soil

Soil	Flavonoids, g%, mean (s.d.)		
	Stage I	Stage II	Stage III
B	0.67 (0.05)	0.54 (0.02)	0.38 (0.02)
C	0.50 (0.02)	0.47 (0.02)	0.49 (0.02)
P	0.48 (0.01)	0.38 (0.02)	0.45 (0.02)

The results of total flavonoids in leaf depending on the same variables are presented in Table I; total flavones could not be detected and quantified on the basis of $AlCl_3$ chelation spectrophotometric method. As for polyphenols and iron, in the case of flavones also there was significant interaction between soil and stage: in the case of B soil the total flavone concentration gradually decreased with stage, in the case of P soil there was a U-shaped relationship, whereas for the C soil the concentration was almost stationary across the three stages. Amounts of 6.6 - 15% have been reported for eriocitrin in mint leaves, with a mean of 10.8%, based on an HPLC method [13]. In commercial samples though, considerably lower amounts have been reported, e.g. between 0.6 and 5.3% for eriocitrin [10]. Our results are considerably lower for total flavonoids, with maximum values of 0.67%; they are, however, not far away from those reported in rutin equivalents (1.41 - 1.53%) other Romanian samples, based on the spectrophotometric method with $AlCl_3$ (Romanian Pharmacopoeia, 10^{th} edition) [4]. Using rutin instead of quercetin (rutin has about twice the molecular weight of quercetin) would lead to a maximum concentration of 0.67 x 2 = 1.34%, a values close to those reported for other samples from the national territory. The difference from the high values reported for eriocitrin in the French samples is striking, though; it is most likely related to the fact that eriocitrin is a flavanone [11] and unlike flavones and flavonols, flavanones do not form stable complexes with aluminium chloride [7]. This fact illustrates the limitations of the methods of estimating total flavone contents based on chelate formation, as these are usually appropriate for flavones and flavonols, but less so for flavanones. It is also interesting that for the aerial parts of *Origanum majorana* L. (*Lamiaceae*) a different pattern of total polyphenol and flavone variation has been reported by other authors using a similar staging as in this paper, with a maximum level at the late or prefloral vegetative stage [6] (whereas in our data at the blooming initiation, which may be considered as roughly equivalent to late or prefloral vegetative stage, the level of total polyphenols and flavones tended to be often minimal and sometimes intermediate, never maximal). In shoots of *Crithmum maritimum* L. (*Apiaceae*) a contrasting pattern was reported between the evolution of total polyphenols and that of total flavonoid contents: total polyphenols were reported to increase from the vegetative to the flowering periods, whereas the flavonoids followed a contrary pattern, decreasing from the vegetative to the blooming stage (these authors only used two stages, vegetative and flowering) [21].

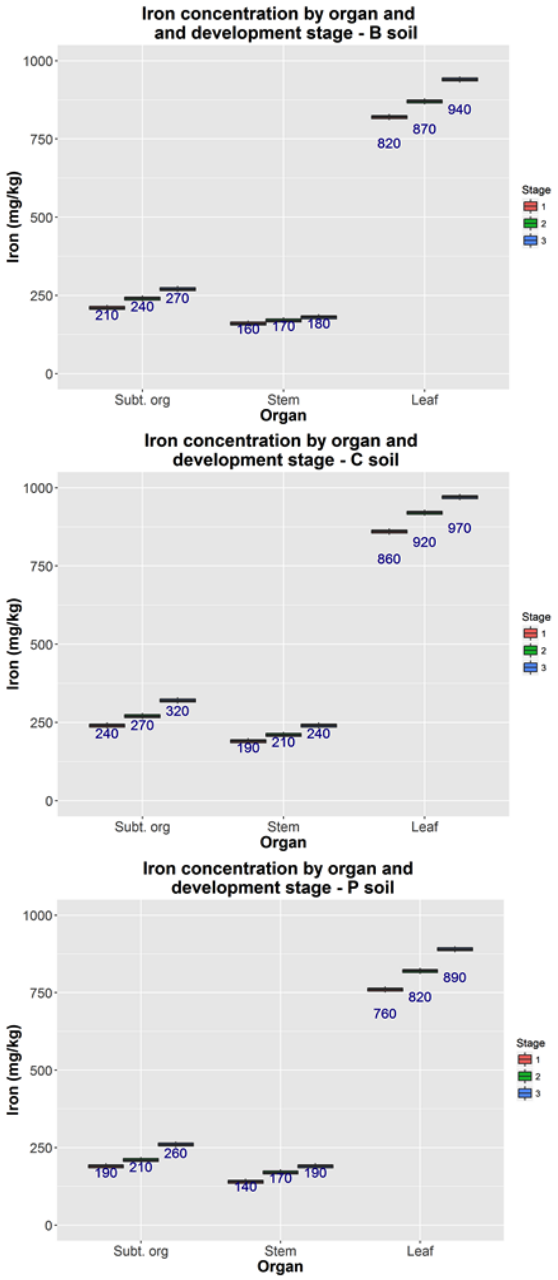

Figure 2.

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).

The contents in iron and other minerals by growth stage and type of soil are shown in Figure 2.

For all three organs (subterraneous ones, stems and leaves) the iron concentration varied significantly among the three stages and types of soil ($p < 0.001$ for each factor). For each type of soil it increased with the development stage ($p < 0.001$) (Figure 2), but the slope was different for each one, with interactions ($p < 0.001$) between soil type and development stage. In the limited literature available on the topic, different patterns have been reported on the variation of iron by development stage. In the leaves of *Prunus avium* (L.) L. (sweet cherry), *Rosaceae* [37] and in leaves or roots of *Phaseolus vulgaris* L. (common beans), *Fabaceae* [5], somewhat similarly increasing trends have been reported; in the leaves, stems and roots of *Medicago sativa* L. (alfalfa), *Fabaceae*, iron concentration was of contrary reported to decrease along three stages [25], whereas in leaves or roots of *Lactuca sativa* L. (lattuce), *Asteraceae*, a U-shaped variation of iron concentrations across three development stages was found [29]. Although we have selected and evaluated the influence of three types of soils, each soil clusters additional variables acting as confounding factors from a statistical standpoint (such as climate and environment, pests etc.), and thus the differences seen between the three soils do not necessarily reflect only soil contribution, but possibly additional external variables. The highest iron contents were recorded in leaves and the lowest in stems, although in the scientific literature it has been estimated that iron content tends to be higher more frequently in roots rather than in leaves, but cases of higher iron contents in leaves *vs.* roots have also been reported [3].

No significant correlation was found between iron and total polyphenol concentration, neither globally, nor for each of the organs analysed separately (globally, Pearson $r = -0.097$, $p = 0.391$; Spearman $r = 0.20$, $p = 0.07$) and the same holds true for the relationship between flavone and iron concentrations (globally, Pearson $r = -0.25$, $p = 0.202$; Spearman $r = -0.23$, $p = 0.242$). Not unexpectedly, a monotonic but limited correlation was recorded between the total polyphenols and flavonoids (Spearman $r = 0.42$, $p = 0.029$; Pearson $r = 0.30$, $p = 0.084$). This expresses the fact that flavonoids are only a part of the polyphenols biosynthesized in a herbal product, and even among flavonoids the aluminium chloride chelation method is only able to quantify a part of the total flavonoids.

Conclusions

The concentration of polyphenols and flavonoids is influenced significantly by both soil and development stage in *Mentha x piperita* L. The highest total polyphenol concentration was detected in roots and subterraneous organs, whereas the highest total flavonoid contents was measured in leaves, being under the level of detection and quantification in stems and roots. The iron contents tended to increase at least monotonically if not linearly in all three organs, with a higher level in leaves than in roots. No significant correlation was found between the levels of total polyphenols or flavonoids and iron, but a limited monotonic relationship was found between polyphenol and flavonoid concentrations.

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References

1. *** Romanian Pharmacopoeia, ed. X, Ed. Medicală, Bucharest, 1993: 835, (available in Romanian).
2. *** European Pharmacopoeia, 9th edition. Council of Europe, Strassbourg, 2016: 37.
3. Ancuceanu R., Dinu M., Hovaneţ M.V., Anghel A.I., Popescu C.V., Negreş S., A Survey of Plant Iron Content-A Semi-Systematic Review. *Nutrients*, 2015; 7(12): 10320-10351.
4. Aprotosoaie A.C., Răileanu E., Trifan A., Cioanca O., The polyphenolic content of common *Lamiaceae* species available as herbal tea products in Romanian pharmacies. *Rev. Med. Chir. Soc. Med. Nat. Iasi*, 2013; 117(1): 233-237.
5. Ayala-Vela J., Guía-González M., Espinosa-Huerta E., Acosta-Gallegos J.A., Guzmán-Maldonado S.H., Mora-Avilés M.A., Iron Content and Ferritin Gene Expression in Common Bean (*Phaseolus vulgaris* L.). *Agric. Tech. Mex.*, 2008; 34(4): 481-489.
6. Baâtour O., Tarchoun I., Nasri N., Kaddour R., Harrathi J., Drawi E., Mouhiba B.N.A., Marzouk B., Lachaâl M., Effect of growth stages on phenolics content and antioxidant activities of shoots in sweet marjoram (*Origanum majorana* L.) varieties under salt stress. *Afr. J. Biotechnology*, 2012; 11(99): 16486-16493.
7. Chang C.C., Yang M.H., Wen H.M., Chern J.C., Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.*, 2002; 10(3): 178-182.
8. Chizzola R., Michitsch H., Franz C., Monitoring of metallic micronutrients and heavy metals in herbs, spices and medicinal plants from Austria. *Eur. Food Res. Technol.*, 2003; 216: 407-411.
9. Dorman H.J., Koşar M., Başer K.H., Hiltunen R., Phenolic profile and antioxidant evaluation of *Mentha x piperita* L. (peppermint) extracts. *Nat. Prod. Commun.*, 2009; 4(4): 535-542.
10. Fecka I., Turek S., Determination of water-soluble polyphenolic compounds in commercial herbal teas from *Lamiaceae*: peppermint, melissa, and sage. *J. Agric. Food Chem.*, 2007; 55(26): 10908-10917.

11. Ferreira P.S., Spolidorio L.C., Manthey J.A., Cesar T.B., Citrus flavanones prevent systemic inflammation and ameliorate oxidative stress in C57BL/6J mice fed high-fat diet. *Food Funct.*, 2016; 7(6): 2675-2681.
12. Fox J., Weisberg S., An {R} Companion to Applied Regression, Second Edition. Sage, Thousand Oaks CA, 2011, www.socserv.socsci.mcmaster.ca.
13. Guédon D.J., Pasquier B.P., Analysis and Distribution of Flavonoid Glycosides and Rosmarinic Acid in 40 *Mentha X piperita* Clones. *J. Agric. Food Chin.*, 1994; 42: 679-684.
14. Hadjmohammadi M., Karimiyan H., Sharifi V., Hollow fibre-based liquid phase microextraction combined with high-performance liquid chromatography for the analysis of flavonoids in *Echinophora platyloba* DC. and *Mentha piperita*. *Food Chem.*, 2013; 141(2): 731-735.
15. Harrell F.E.Jr., Dupont C., Hmisc: Harrell Miscellaneous. 2016; R package version 3.17-4, www.CRAN.R-project.org/package=Hmisc.
16. Hoffmann B.G., Lunder L.T., Flavonoids from *Mentha piperita* Leaves. *Planta Med.*, 1984; 50(4): 361.
17. Hossain M.A., Al-Hdhrami S.S., Weli A.M., Al-Riyami Q., Al-Sabahi J.N., Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman. *As. Pac. J. Trop. Biomed.*, 2014; 4(Suppl 1): S368-372.
18. Hur M.H., Park J., Maddock-Jennings W., Kim D.O., Lee M.S., Reduction of mouth malodour and volatile sulphur compounds in intensive care patients using an essential oil mouthwash. *Phytother. Res.*, 2007; 21(7): 641-643.
19. Hurrell R.F., Reddy M., Cook J.D., Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages. *Br. J. Nutr.*, 1999; 81(4): 289-295.
20. Inoue T., Sugimoto Y., Masuda H., Kamei C., Anti-allergic effect of flavonoid glycosides obtained from *Mentha piperita* L. Biol. *Pharm. Bull.*, 2002; 25(2): 256-259.
21. Iancu C., Cioancă O., Mircea C., Mocanu M., Hăncianu M., *Pelargonium sp.*: characterization of the polyphenols and their biological potential. *Farmacia*, 2016; 64(3): 333-338.
22. Kabir M.G., Rahman M.M., Ahmed N.U., Fakruddin M., Islam S., Mazumdar R.M., Antioxidant, anti-microbial, toxicity and analgesic properties of ethanol extract of *Solena amplexicaulis* root. *Biolog. Res.*, 2014; 47(1): 36.
23. Liu X., Sun Z.L., Jia A.R., Shi Y.P., Li R.H., Yang P.M., Extraction, preliminary characterization and evaluation of *in vitro* antitumor and antioxidant activities of polysaccharides from *Mentha piperita*. *Int. J. Mol. Sci.*, 2014; 15(9): 16302-16319.
24. Lozak A., Soltyk K., Ostapczuk P., Fijalek Z., Determination of selected trace elements in herbs and their infusions. *Sci. Tot. Environ.*, 2002; 289(1-3): 33-40.
25. Marković J., Štrbanović R., Cvetković M., Anđelković B., Živković B., Effects of growth stage on the mineral concentrations in alfalfa (*Medicago sativa* L.) leaf, stem and the whole plant. *Biotechnol. Anim. Husbandry*, 2009; 25(5-6): 1225-1231.
26. McKay D.L., Blumberg J.B., A review of the bio-activity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother. Res.*, 2006; 20(8): 619-633.
27. Ozcan M.M., Akbulut M., Estimation of minerals, nitrate and nitrite contents of medicinal and aromatic plants used as spices, condiments and herbal tea. *Food Chem.*, 2008; 106(2): 852-858.
28. Pena E.A., Slate E.H., gvlma: Global Validation of Linear Models Assumptions. 2014. R package version 1.0.0.2. www.CRAN.R-project.org/package=gvlma.
29. Pillay V., Jonnalagadda S.B., Elemental uptake by edible herbs and lettuce (*Latuca sativa*). *J. Environ. Sci. Health Part B*, 2007; 42(4): 423-428.
30. Pinheiro J., Bates D., DebRoy S., Sarkar D., R Core Team. nlme: Linear and Nonlinear Mixed Effects Models. 2016. R package version 3.1-128, www.CRAN.R-project.org/package=nlme.
31. Pytlakowska K., Kita A., Janoska P., Połowniak M., Kozik V., Multi-element analysis of mineral and trace elements in medicinal herbs and their infusions. *Food Chem.*, 2012; 135(2): 494-501.
32. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, 2016, www.R-project.org.
33. Raczuk J., Biardzka E., Daruk J., *Zawartosc Ca, Mg, Fe i Cu w wybranych gatunkach ziól i ich naparach* (The content of Ca, Mg, Fe and Cu in selected species of herbs and herb infusions). *Rocz. Panstw. Zakl. Hig.*, 2008; 59(1): 33-40.
34. Razic S.S., Dogo S.M., Slavkovik L.J., Multivariate characterization of herbal drugs and rhizosphere soil samples according to their metallic content. *Microchem. J.*, 2006; 84(1-2): 93-101.
35. Romero M.C., Navarro M.C., Martín-Sánchez J., Peppermint (*Mentha piperita*) and albendazole against anisakiasis in an animal model. *Trop. Med. Int. Health*, 2014; 19(12): 1430-1436.
36. Rubio C., Lucas J.R., Gutiérrez A.J., Glez-Weller D., Pérez Marrero B., Caballero J.M., Revert C., Hardisson A., Evaluation of metal concentrations in mentha herbal teas (*Mentha piperita*, *Mentha pulegium* and *Mentha* species) by inductively coupled plasma spectrometry. *J. Pharm. Biomed. Anal.*, 2012; 71: 11-17.
37. Sanchez-Alonso F., Lachica M., Seasonal trends in the elemental content of sweet cherry leaves. *Commun. Soil Sci. Plant Anal.*, 1987; 18(1): 17-29.
38. Shah N.A., Khan M.R., Sattar S., Ahmad B., Mirza B., HPLC-DAD analysis, antioxidant potential and anti-urease activity of *Asparagus gracilis* collected from District Islamabad. *BMC Complement. Altern. Med.*, 2014; 14: 347.
39. Sroka Z., Fecka I., Cisowski W., Antiradical and anti-H_2O_2 properties of polyphenolic compounds from an aqueous peppermint extract. *Z. Naturforsch C*, 2005; 60(11-12): 826-832.
40. Suliburska J., Kaczmarek K., Herbal infusions as a source of calcium, magnesium, iron, zinc and copper in human nutrition. *Int. J. Food Sci. Nutr.*, 2012; 63(2): 194-198.
41. Toiu A., Vlase L., Arsene A.L., Vodnar D.C., Oniga I., LC/UV/MS profile of polyphenols, antioxidant

and antimicrobial effects of *Ajuga genevensis L.* extracts. *Farmacia*, 2016; 64(1): 53-57.

42. Tanase C., Volf I., Popa I.V., Enhancing copper and lead bioaccumulation in rapeseed by adding hemp shives as soil natural amendments. *J. Environ. Engin. Land. Manag.*, 2014; 22(4): 245-253..
43. Venables W.N., Ripley B.D., Modern Applied Statistics with S. Fourth Edition. Springer, New York, 2002.