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The determination of iron levels in Menthae tea (*Mentha piperita* L.)

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Abstract: Menthae tea (*Mentha piperita* L.) is one of the most widely consumed herbal teas. This tea is recognized as a drink that may have several health benefits, primarily due to the presence of nutritional elements especially essential micro and ultramicro elements. In this study, we investigated the content of iron in mentha tea samples found in a local market in Sarajevo. The preparation of the samples was done by dry digestion in triplicate while levels of iron were analyzed by spectrophotometry. The amounts of iron were ranged from 275.6 mg Fe/kg to 354.6 mg Fe/kg. The used spectrophotometric method is simple and sensitive method that can be applied for the determination of total Fe content in plant material.

INTRODUCTION

People around the world use medicinal plants as herbal tea and for the health reasons. World Health Organization (WHO), reported that, about 80% of population in peripheral communities use only medicinal herbs for the treatment of many diseases. Chemical compositions of different medicinal and aromatic plants contain proteins, lipids, carbohydrate and mineral elements. Because of that, many plants are consumed as spices, dietary supplements, or serve as herbal teas. Recently, it has been a lot of scientific interest for the determination of composition i.e. nutrient contents of plants, especially for the determination of the micro and ultra microelements in plants (Szentmihályi et al., 2008; Remigius, 2012; Mihaljev et al., 2014; Živkov-Baloš et al., 2014). Additionally, minerals and trace metals are partially recognised for their medicinal and nutritional properties, as well as their toxic ones. Microelements are known as nutrients for which there are RDA (Recommended Dietary Allowances) values available. Based on this set values consuming 1L of tea, in some cases will cover 10% or more of the daily requirement for several elements. However rganic components of the plant parts may change and the microelement content of the different parts of plant drugs are also different (Szentmihályi et al., 2008; Gogoasa et al., 2013; Mihaljev et al., 2014). Therefore, the phytotherapeutic effect of medicinal plants may also differ according to the plant species and plant parts.

Among the other essential elements, iron is an indispensable microelement for living organisms because of its participation in metabolic processes, such as transport of oxygen, DNA synthesis, and electron transfers. It is also possible that medicinal plant raw materials may supply this element for humans with iron deficiency. This issue is extremely important for pregnant and lactating women. Several medicinal plants are also traditionally used against anemia.

Number of studies focusing on determination of concentrations of micro and ultra-microelements in medicinal plants; have established that iron occur in the range of concentration from several tens to several hundreds/thousands of mg/kg of dry plant weight, and that its level in many cases depends on genetic factors and on the morphological part of a plant. The levels of essential elements in plant vary according to the geographical region, geochemical soil characteristics, and the ability of plants to selectively accumulate some of these elements. Generally, these elements are absorbed through the root systems and dispersed throughout the plant body (Korfali et al., 2013).

Mentha piperita L. (Family: Lamiaceae), commonly known as peppermint, is an important medicinal herb worldwide. "Medicinal plant of the year 2004"; oldest known medicinal plant species in Eastern and Western traditions although first described in 1753 by Carolus Linnacus,It is used as flavoring agent, in cosmetics preparations , and as pharmaceutical products amongst others. The trace elements present in Menthae may play a direct or indirect role in their biological activities: anti-inflammatory, antioxidant, antimicrobial, antifungal activities. Provided studies show antioxidant and antimicrobial properties of the Menthae leaves which are locally available (Gonçalves et al., 2009; Kızıl et al., 2010; Pramila et al., 2012; Saeed et al., 2014; Siddig et al., 2015). Some investigators found that its antifungal activity is comparable to that of synthetic fungicides. As a significant trace element, iron is necessary for all living organisms and essential element in cell metabolism (involved in photosynthesis, respiratory, etc.). Since elements are able to pass through different membranes, essential elements get into the cells and organs of human body that cases favorable or non-favorable processes. Therefore, increasing focus on the importance of dietary minerals in the prevention of disease justifies need for more serious studies on the mineral content of plants (Saeed et al., 2014; Stanojkovic et al., 2015).

The aim of this study is to determine of total content of iron in some brand Menthae tea samples, using dry digestion as method for preparation herbal samples (biological materials) and spectrophotometric analysis as method of quantification.

EXPERIMENTAL

Biological material

Plant samples (mint teas-Menthae folium) were taken from different manufactures from a local market in Sarajevo: PAK NANA (Bosnia and Herzegovina), 1000 CVET (Slovenia) and Pharmamed (Bosnia and Herzegovina).

Sampling and sample preparation

The preparation of Menthae tea samples was carried out by dry digestion. The herbal samples were prepared with mass of 1.0000± 0.0002 g in crucible and heated during 4 hours at temperature of 500^{0}C in furnace. After cooling, ashes were dissolved in concentrated nitric acid (1ml), crucible washed with mixture of nitric and hydrochloric acids (concentration of 0.05 moldm^{-3}), and filtrated (blue filtered paper) in volumetric flask (100 cm^3), then diluted to mark with same mixture of acids. Digestion of each tea samples were done in triplicate.

Reagent and standard solutions

All reagents were analytical grade (p.a.) while distilled water (Milli-Q, Millipore), used for samples dilution and labware washing.

Instruments

- Spectrofotometer "Spectronic Genesys 2" (Spectronic Instruments, Milton Roy Company, Champaign, Illionois, USA)
- The device for the production of ultrapure water (Arium® 611, Sartorius Mechatronics, Germany)
- Analytical balance (AX 250 Delta Range®, Mettler Toledo Inc., USA), Sartorius analytical balance
- Technical scales (BL 1500, Sartorius Mechatronics, Germany)
- Magnetic mixer with heater (Heidolph Instruments GmbH & Co.KG Lab Equipment Sales Walpersdorfer Str. 12 D-91126 Schwabach, Germany)
- Furnace (Nabertherm 30-3000°C, Lilienthal, Germany)

Spectrophotometric method

Spectrophotometric method was performed with a *Spectronic Genesys 2* spectrophotometer. Solutions of each of nine samples were red to red pale color. Before spectrophotometric analysis, intensity of color, increases by the addition of a reagent, potassium thiocyanate (5 moldm^{-3}), for complexation of iron ions and formation of red complex with different composition, from $[FeSCN(H_2O)_5]^{2+}$ to $[Fe(SCN)_6]^{3-}$, and intensity of color solutions. Potassium thiocyanate is an inexpensive, available reagent that forms iron (III)-ions with a stable complex. This reaction is favorable in acidic medium and takes place at a temperature of 50^{0}C, and with acid that will not complexe iron (III) ions. For this reaction nitric acid was used, with concentration of 5 moldm^{-3}.

The calibration solutions were prepared over a concentration range of 1.40 and 69.81 μg/cm^3 Fe .Absorbance was measured at 481,0 nm for working and analyzed solutions (the calibration curves demonstrated adequate linearity, R^2 = 0.9999). Calibration curve is presented in Figure 1.

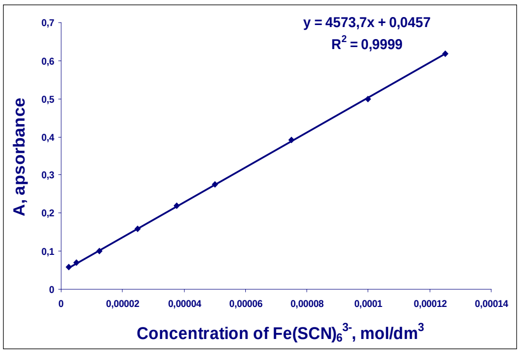

Figure 1. The calibration curve for spectrophotometric analysis of Menthae tea samples

Statistical analysis

All the assays were carried out in triplicates. The experimental results were expressed as mean ± standard deviation. The data were analyzed using Microsoft Office Excel.

RESULTS AND DISCUSSION

Preparation of tea samples was done by dry digestion, while the quantitative analysis and determination of the concentration of iron was performed spectrophotometrically, using the thiocyanate method. Results showing concentrations of iron (total content of iron of leaf of mentha herbs) in three different Menthae tea samples, produced by different manufactures and purchased at a local market in Sarajevo are presented in Table I. The content of studied metal is expressed in mg/kg of dry matter (d.m.). Range of iron levels varies from 275.6 to 354.6 mg/kg d.m. with average value of 320.8 mg/kg d.m.

Table 1. Content of iron in Menthae tea samples
Data are expressed as mean ±SD. A, Apsorbance; d.m., dry mass

Tea sample	A	mean of Apsorbance	mean of Concentration $[Fe(SCN)_6]^{3-}$ (mol/dm^3)	Content of Fe (mg/kg of d.m.)
Sample 1		0.6273	$1.27\cdot10^{-4}$	354.6±6.8
I	0.701			
II	0.615			
III	0.566			
Sample 2	0.487	0.4973	$9.87\cdot10^{-5}$	275.6±6.6
I				
II	0.437			
III	0.568			
Sample 3	0.577	0.5893	$1.19\cdot10^{-4}$	332.3±5.7
I				
II	0.540			
III	0.651			

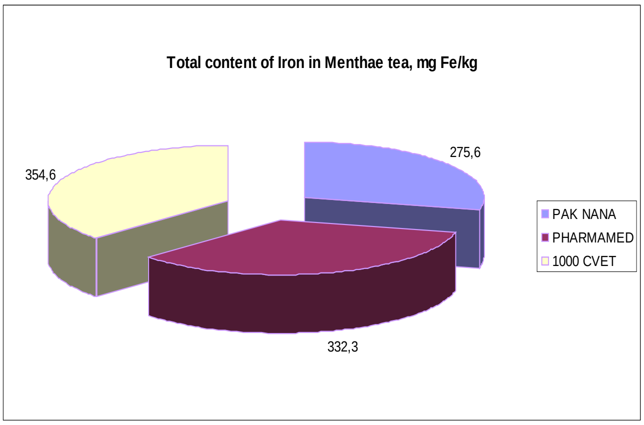

Figure 2. The total content of iron in analyzed Menthae tea samples

Our results are in line to previously provided studies and demonstrated that levels of iron in most of mentha species are highest when compared to other mineral elements. Concentrations of iron (mg Fe/kg of d.m) were from 358 mg Fe/kg, 293 mg Fe/kg, 260 mg Fe/kg and 489 mg Fe/kg, to 1068 mg Fe/kg and 11841mg Fe//kg (Arzani et al., 2007; Bielicka-Giełdoń and Ryłko, 2013; Ebrahimzadeh et al., 2011; Szymczycha-Madeja et al., Maghrabi, 2014; Faiku and Haziri, 2015, respectively) or 129 mg Fe/kg and lowest when compared to mean of 320.8 mg Fe/kg, obtained in this study. Data obtained in study Živkov-Baloš et al., (2014) for content of iron in *Mentha piperita* of 274.83 mg Fe/kg d.m. are similar to our results for sample tea *1*.

Contents of microelements in medicinal plants are influenced by genetically-determined properties of a plant as well as by external factors, including geographic location, soil type and profile, fertilization, availability of water, pollution by pesticides or dusts, and gases. Moreover, highest iron a concentration was found from locality were menthe plant grows and cultivated as organic species (Faiku and Haziri, 2015; Maghrabi, 2014). Concentrations of some elements as well as iron are also affected by technological processes, duration and storage conditions.

Leaves of mentha plant are frequently used in herbal teas and for culinary purpose to add flavor and aroma. Also, mentha spices are rich sources of iron among others trace elements which play important role in human nutrition.

Mentha as medicinal plant and raw material may be used as a supplement in cases of iron deficiency. However, it is also known from the literature (Lozak et al., 2002; Konieczynski and Wesolowski, 2008; Szymczycha-Madeja et al., 2013; Mirzaei et al., 2015), that only a small percentage of iron present in medicinal plants is bioavaible, when considering its water-soluble species.

Taking this into account, total content concentration of iron in some medicinal plants as well as in menthe depends on or is influenced by pH. At concentration of iron species in aqueous solutions, at the characteristic pH of saliva (pH6.5), stomach juice (pH1.2), and intestine juice (pH7.8) could help to answer the questions whether and what amounts iron species can be potentially absorbed in specific segments of the digestive system of humans. Only soluble iron can be absorbed in the human digestive system, thus, only a fraction of iron can be treated as being bioavailable. It has been shown that pH has significant effects on the determined species. Comparing results of iron species determined in all solutions with different pH values, it can be seen that pH significant impact on the amounts of bioavailable iron. These results indicate that only the fractions of iron detected in the particular solutions can be considered bioavailable iron in the neutral aqueous solutions. The reason for this is probably the complex composition of plant material, in which iron is bound by various ligands. Also, brewing time had significant effect on the percentage of microelement extraction to the aqueous phase, such in the case of iron (Dormana et al., 2009; Zijp et al., 2010; Sembratowicz et al., 2014).

Some trace elements are essential for normal growth and functioning of living organisms, but only at low concentrations. They catalyze many biochemical reactions occurring in the organisms that are involved in the formation of red blood cells, hormones, and vitamins, as well as take part in the processes of photosynthesis and the creation of pigments, respiration, oxidation, and reduction. Some of these elements are important in metabolism, and are part of the bone and tissues of living organisms, and participate in the functioning of neural systems. But in too high doses these metals can be toxic (Gonçalves, 2009; Denys, 2012; Bielicka-Giełdoń et al., 2013; Mirzaei et al., 2015).

Phytopharmaceuticals are gaining popularity worldwide; however, cases of adverse effects and drug interactions have also increased. One reason is in the high metal content both as ingredients but also as contaminants. Metal monitoring in food, like herbal teas, provides basic information on safety aspects in regulatory processes as well as nutritional values. Metal contents varied significantly, depending on the stores the products were purchased in and on tea packaging (loose leaves versus tea bags). Mentha plants are mainly used for treatment of disorders of gastrointestinal tract. They have also been reported to have antioxidant, anti-inflammatory, antimicrobial, analgesic and anticarcinogenic effects (Rubio et al., 2012; Prabu et al., 2015; Siddig et al., 2015). Herbalists consider peppermint as an astringent, antiseptic, antiemetic, carminative, analgetic. The plant extract that possesses radioprotective, antioxidant, anticancerogenic, antitumorgenic, antiandrogenic, antiallergic, antispasmolic properties amongst others. Plant extract can, also, reduce the arsenic-induced toxicity; reduce glucose, total cholesterol, LDL-chol, and triglycerides levels (in diabetic rat). In study Barbalho et al., (2011) results indicate that *Mentha piperita* can be used for therapeutic and preventive affecting biochemical profile, blood pressure and body mass index in humans.

Medicinal plants are the raw material for many herbal formulations and popular supplements. The use of herbal medicines has been on the rise in recent years due to their low prices. The last few decades have witnessed a rapid development in the diet studies focused on the determination of trace elements, which reflect their role in human health and nutrition. Deficiency, excess or imbalance of trace element intake into human body may result in various diseases.

CONCLUSION

The determination of element content in medicinal plant drugs and extracts has several important aspects. Since elements are able to pass through different membranes, essential elements enter into the cells and organs of human body that might be favorable or non-favorable processes. Among the other essential elements, iron is the most important microelement for living organisms because of its participation in metabolic processes, such as transport oxygen, DNA synthesis, and electron transfers.

In our study, all tested Menthae tea samples showed high iron levels, which indicate that dry digestion as method for preparation and spectrophotometric analysis, are applicable for detection and determination of iron concentrations in biological materials.

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Summary/Sažetak

Čaj od mente (*Menthae piperita* L.) je jedan od najčešće konzumiranih biljnih čajeva. Ovaj je čaj prepoznat kao napitak sa više zdravstvenih benefita, prije svega usljed prisustva nutritivnih elemenata posebno esencijalnih mikro i ultramikro elemenata. U ovoj studiji ispitali smo sadržaj željeza u uzorcima čajeva od mente iz lokalnih marketa u Sarajevu. Pripremanje uzoraka provedeno je suhom digestijom u triplikatu dok je sadržaj željeza analiziran spektrofotometrijski. Količina željeza bila je u intervalu od 275.6 mg Fe/kg do 354.6 mg Fe/kg. Primjenjena spektrofotometrijska metoda je jednostavna i osjetljiva te se može koristiti za određivanje ukupnog sadržaja željeza u biljnom materijalu.