Determination of mineral element content of *Pelargonium roseum* plant by ICP-MS

Carmen Gâlea¹, Gabriel Hancu^{2*}, Attila Csiszer³, Csengele Monika Jeszenszky⁴,
Enikő Barabás⁵

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University "Lucian Blaga", Sibiu, Romania

² Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy,
Tîrgu Mureş, Romania

³ National Institut of Public Health, Tîrgu Mureş, Romania

⁴ Faculty of Medicine, University of Medicine and Pharmacy, Tîrgu Mureş, Romania

⁵ Department of Botany and Cell Biology, Faculty of Pharmacy, University of Medicine and Pharmacy,
Tîrgu Mureş, Romania

Received: 6 December 2015; Accepted: 10 January 2016

Abstract

Microelement content of different vegetative parts of *Pelargonium roseum* was evaluated by inductively coupled plasma – mass spectrometry (ICP-MS) technique. The analytical method allowed the determination of 20 elements (Li, Be, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Ag, Cd, In, Ba, Tl, Pb, Bi). Samples from different vegetative parts (roots, lignified stems, green stalks, leafs, blooms) were analyzed and compared with soil samples. The vegetative part of the plant contained significant quantities of manganese, zinc and especially iron. The results can be very useful for the determination of the recommended doses of plant products for use in medicinal plant products considering its nutritional content and pharmacological functions.

Keywords: *Pelargonium roseum*, Microelemental analysis, ICP-MS, Dietary supplements

Introduction

Pelargonium roseum is a species of aromatic hairy herbaceous shrub indigenous to Southern Africa belonging to the *Geraniaceae* family, which was introduced and nowadays is highly cultivated in the Mediterranean part of Europe, Northern Africa and Central America. Sometimes this herb is also called *Pelargonium graveolens*, but despite the double name, it is the same herb - *Rose geranium*. It is called *Rose Geranium*, because this plant has both the characteristics in itself - Rose, because of the scent when it blooms and Geranium, because it is from the same genus

as the common Geraniums (*Geraniaceae*) (Lis-Balchin et al., 1996).

Pelargonium roseum has a woody, straight stem with branches; its leaves are usually alternate, palmate lobed or pinnate, often on long stalks, and sometimes with light or dark pattern; covered with short, rough hairs, which give the plant a strong, pleasant rose-like scent. The erect stems bear five-petaled flowers in umbel-like clusters; the flower has a single symmetry plane (zygomorphic), which distinguishes it from the *Geranium* flower, which has radial symmetry (actinomorphic). It is an annual plant, but when dwarfed and domesticated it becomes perennial. Roots grow up to 60 cm in soil, fresh leaves grow in the whole year if the temperatures are not too low and its flowers

* e-mail: gabriel.hancu@umftgm.ro

blooms actively between June and October (Fig. 1) (Lancu et al., 2013).

It is cultivated mainly by the perfume industry and for aromatherapy and there are subspecies with various other scents. Calming and grounding, *Pelargonium roseum* essential oil helps to reduce feelings of stress and worry; it's balancing effect on the body and mind makes this oil an excellent choice to uplift the mood and promote feelings of well being (Dorman and Deans, 2000; Moyo and van Staden, 2014).

Essential oil of *Pelargonium roseum* is used in herbal medicine for its astringent and chemostatic effect, also it regulates the bloodstream, stimulates the adrenal glands and lymphatic system which in combination with its diuretic properties makes this essential oil excellent in the fight against cellulite and fluid retention in the body (Cavar and Maksimovic, 2012; Dzamic et al., 2014).

The antiseptic, antimicrobial and antifungal properties of *Pelargonium roseum* essential oil were also assessed and evaluated, with encouraging results, when compared with the effects of different largely used antimicrobial substances (Baratta et al., 1998; Dorman and Deans, 2000)

An interesting study evaluated the potential of *Pelargonium roseum*, to uptake and accumulate heavy metals like nickel (Ni), cadmium (Cd) or lead (Pb); the metal content in the extracts from the entire biomass of the plant was estimated using a flame atomic absorption spectrophotometer; the results of the study indicated the efficacy of this plant for decontamination of multiple metal-polluted sites in phytoremediation industry (Mahdiah et al., 2013).

However there are no studies regarding the mineral element content of different parts of the plant. The aim of the

present study was to analyze traces of elements in different parts of the plant (roots, stalks, leaves and flowers), with the purpose of demonstrating the potential for a possible use of this plant in nutritional supplements.

Material and methods

Plant material cultivation and harvesting

Cultivation of *Pelargonium roseum* species was carried out in the herb garden of the University of Medicine and Pharmacy from Tîrgu Mureş, Romania, on a total area of 200 m². The harvest of the aerial parts was performed in 3 stages; in July we harvested the ramifications of stalks with 3-5 leaves, in August the ramifications of well-developed stalks and the third harvest was made at the end of the vegetation period, in September. The roots were harvested at the beginning of October. Throughout the cultivation period no herbicides or chemical fertilizers were used, because we wanted to preserve unspoiled the qualities of the essential oils obtained subsequently.

From the harvested stalks and leaves, a part was dried in order to prepare infusions and for physical and chemical determinations, and another was subject to distillation in order to obtain essential oil.

Samples from roots, lignified stems, green stalks, leafs and blooms were analyzed and the results were compared with the ones obtained from soil samples. The soil samples were taken from the cultivation zone.

Fig. 1. *Pelargonium roseum*.

Reagents

The mineral content of the plant was determined using an ICP-MS method. The technique can be used primarily for the positive ions detection. The elements whose concentration was studied were: lithium (Li), beryllium (Be), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), gallium (Ga), selenium (Se), rubidium (Rb), strontium (Sr), silver (Ag), cadmium (Cd), indium (In), barium (Ba), thallium (Tl), lead (Pb) and bismuth (Bi).

All reagents used for the microwave-assisted digestions: hydrochloric acid (HCl 36% (v/v)), nitric acid (HNO₃ 69% (v/v)) and hydrogen peroxide (H₂O₂ 33% (v/v)) (Merck, Darmstadt, Germany), were of analytical grade. High-purity water (18MΩcm) obtained with a NANOpur water purification system (Barnstead D4745, Thermolyne, USA) was used for standard preparation and sample dilution. Before use, all lab utensils were thoroughly acid cleaned and then rinsed with ultrapure water. All Teflon digestion vessels were previously cleaned in a bath with 10% (v/v) nitric solution for 24h, and then were rinsed with nanopure water, to avoid cross-contamination.

Sample preparation

The green mass subject to ICP determinations was processed by wet digestion method. The dried samples were grounded with a Mixer Grinder, and all the samples were originally stored in closed plastics bags until analysis. Samples of plant material were weighed (0.3 - 0.4 g / sample), introduced in teflon vessels and treated with nitric acid (4 mL), hydrochloric acid (0.25 mL) and hydrogen peroxide (1 mL). The vessels were then covered and left standing 24 hours under a hood, for the full decomposition of the plant material. After that the vessels were introduced in a microwave digestion apparatus for 30 minutes, where mineralization was performed in conditions of high pressure and temperature. The vessels were cooled for one hour and opened in order to evacuate the nitrogen oxides and the nitric acid. The solutions obtained through this process were transferred in calibrated tubes and filled with nanopure water to 15 mL. A further dilution (1:10) was made in order to obtain the final samples analyzed by ICP-MS (Mihaylova et al., 2013).

The closed-vessel microwave digestion of the samples was performed with Microwave Accelerated Reaction System (Anton Paar, Multiwave 3000) (CEM, Mars 5).

ICP-MS analysis

Analysis of the samples was carried out using an ICP-MS Ultramass 700 Varian system, with glass concentric nebulizer and quadropol detection. Argon (purity higher than 99.995%) (Siad SRL, Cluj-Napoca, Romania) was used as plasma and carrier gas.

A multi-elemental ICP standard solution XXI Certi

Pur (Merck, Darmstadt, Germany), containing all the analyzed elements was used for calibration. The external calibration solutions including known concentrations of each target analyte, were prepared by diluting the stock multi-element standard solution in nanopure water containing 1% nitric acid. A blank solution consisting in nanopure water containing 1% nitric acid was used for the calibration curve. The nonspectral matrix effects associated to the ICP-MS measurements were resolved by the addition of internal standards to all of the used solutions (blank, calibration and sample). The standard solution was prepared by diluting single elemental stock solutions with nanopure water containing 1% nitric acid and up to 50 µg/L rhodium, terbium, yttrium and scandium. The calibration coefficients for all calibration curves were at least 0.999.

The isotopes used in this study were the following: Li-7, Be-9, Cr-52, Mn-55, Fe-57, Co-65, Zn-66, Ga-71, Se-78, Rb-85, Sr-88, Ag-107, Cd-111, In-115, Ba-137, Tl-205, Bi-209. The isotopes were chosen after careful evaluation of all possible isotopes, their abundances, potential interferences (meaningful in the analysis of plants) and the appropriate corrections were introduced.

The samples used for the trace element determination were prepared as follows: 1 mL solution obtained by wet digestion was filled with 8.70 mL of nanopure water and 0.1 mL internal standard (rhodium 4 µg/l and 0.2 mL isopropanol), thus obtaining a sample of 10 mL. This sample solution was introduced in the apparatus using a peristaltic pump, and brought in the form of aerosol by an argon plasma nebulizer.

Results and Discussion

The results of the microelemental analysis of the vegetative parts of the *Pelargonium roseum* plant are presented in Table 1.

The amounts of Li, Be, Cr, Co, Ga, Se, Ag, Cd, In, Tl, Bi were quantified in µg/kg while the amounts of Mn, Fe, Ni, Cu, Zn, Rb, Sr, Ba, Pb were quantified in mg/kg.

It is remarkable that the values obtained in the case of Fe analysis are significant; in green stalks Fe concentration was found to be 1.21 g/kg, whereas in leaves it was 0.66 g/kg (Fig. 2).

The Fe content is strongly influenced by pH and the redox potential of the environment, so that an alkaline soil favors Fe precipitation while an acidic soil determines its solubilization (Merian et al., 2008). The soil on which we cultivated *Pelargonium roseum* was a clayey soil with a pH of 7.63 and a Fe composition of 36.84 g/kg. The roots of the plant can be considered as Fe accumulator (11175 mg/kg), but usually Fe has a relatively low mobility in plant tissues. The lower content of Fe in the stem (314.7 mg/kg) and leafs (606 mg/kg) can be explained due to the involvement of Fe in the photosynthesis process.

The Fe daily needs of a human body is 8 mg/day for young women and 6 mg/day for men and for women over

Table 1. Microelement content of the vegetative parts of *Pelargonium roseum*

Vegetative parts	Microelement content (µg/kg)										
	Li	Be	Cr	Co	Ga	Se	Ag	Cd	In	Tl	Bi
Roots	1073	59	5472	650	270	490	22	640	39	29	3
Lignified stems	59	36	744	221	31	-	17	199	2	9	6
Green stalks	80	50	940	119	45	424	10	164	8	9	150
Leafs	267	25	1584	1232	79	-	98	8	23	20	7
Blooms	227	161	1974	493	96	4161	-	20	12	40	45

Vegetative parts	Microelement content (mg/kg)									
	Mn	Fe	Ni	Cu	Zn	Rb	Sr	Ba	Pb	
Roots	40.8	1175.6	3.5	19.7	62.5	5.4	74.1	24.8	156.5	
Lignified stems	29.7	314.7	1.1	6.2	74.6	2.5	30.8	11.7	1	
Green stalks	39.4	1213.9	1.2	4.5	54.9	6	54.8	11.2	0.8	
Leafs	53.3	606	2.1	4.8	31.5	3.2	40.2	16.1	5.5	
Blooms	45.7	967.2	2.2	8.3	42.2	4.8	36.3	28.5	3	

50 years, therefore vegetative parts of *Pelargonium rosuem* can be a valuable source of Fe.

Amounts comparable with those found in the soil were found for Sr; as the roots contained a quantity of Sr (74.1 mg/kg), which exceeded the quantity found in the soil (54.82 mg/kg) (Fig. 3).

Previous studies show a correlation between the ratio of Sr and Ca from plants, both elements being taken up from soil in similar proportions. Although Ca was not determined from the vegetative parts of the plant, being present over the concentration range on which the determination was made; traces of Ca have been found at a morphological examination of the plant when calcium oxalate

crystals were revealed (especially in roots). To date there are no eloquent studies regarding the essentiality of Sr for plants, animals or people (Merian et al., 2008).

Mn plays an essential part in plant metabolism, its function being correlated with redox processes when changes its valence from Mn²⁺ to Mn⁴⁺. It plays an important role in the photosynthesis process, both as oxygen generator and participating in the electronic transport associated to this process (Merian et al., 2008).

The active role, which Mn plays in photosynthesis, explains its presence in high quantities in leafs (53.3 mg/kg); while the smaller quantities were identified in the stems (29.7 mg/kg), where the presence of chlorophyll is low-

Fig. 2. Fe concentration in different vegetative parts of *Pelargonium roseum*.

Fig. 3. Sr concentration in different vegetative parts of *Pelargonium roseum*.

Fig. 4. Mn concentration in different vegetative parts of *Pelargonium roseum*.

er (Fig. 4).

Mn plays an important role in human metabolism as constituent and activator of different enzymatic systems. The Mn daily need of the human body is between 2-5 mg/day, therefore vegetative parts of *Pelargonium roseum* can be a source of Mn.

Relatively high concentrations of Zn were found in the vegetative parts of the plant, the highest concentrations were found in stems (74.6 mg/kg) and roots (62.5 mg/kg) in comparison with the soil content (170 mg/kg) (Fig. 5).

Zn is considered an essential element for living organisms, entering in the composition of more than 300 enzyme and proteins, which take part in all major metabolic pro-

cesses.⁹ The Zn daily needs of a human body is 4.6 mg/day for men and 3.8 mg/day for women, therefore *Pelargonium roseum* can be useful in completing the daily needs of Zn.

High quantities of Se were found in the blooms (4161 µg/kg) in comparison with soil (490 µg/kg) (Fig. 6). The Se content from soil is not direct proportional with its biodisponibility towards plants (Merian et al., 2008).

Se is involved in various enzymatic mechanisms, having also a protective role against toxins and carcinogens from the environment. The Se daily needs of a human body is around 20 µg/day; excepting its blooms *Pelargonium roseum* contains relatively small amounts of Se; but it should be noted that Se biodisponibility in the case of

plants is much higher to that of the nutritional supplements, which recommends medicinal plants usage in order to supplement the shortage of this element (Merian et al., 2008).

The content of Li, Rb, Be, Ba, Cr, Co, Ni, Cu, Cd, Pb, In, Tl in the vegetative parts of the plant were much lower than their content in the soil.

All concentrations were within the recommended daily allowance (RDA) limits. RDA, the average daily dietary intake level, is expected to be sufficient to meet the nutrient requirements of all healthy individuals (World Health Organization, 1991; World Health Organization, 1998).

Human dietary micronutrients are required by humans in very small amounts, including at least 14 trace elements (As, B, Cr, Cu, F, I, Fe, Mn, Mo, Ni, Se, Si, V, Zn). The rec-

ommended daily intake of the micronutrient trace elements is of the order of: mg per day for B, Cu, F, Fe, Mn, Zn and µg per day for As, Cr, I, Mo, Ni, Se, Si, V.

These results, therefore, would suggest that consumption of *Pelargonium roseum* powder can provide users with some of the essential minerals that the human body requires for optimum function.

Conclusions

The knowledge of the elemental composition of plants is important for a variety of scientific areas – plant physiology, agriculture, environmental monitoring, medicine or

Fig. 5. Zn concentration in different vegetative parts of *Pelargonium roseum*.

Fig. 6. Se concentration in different vegetative parts of *Pelargonium roseum*.

pharmacy; as plants usually uptake nutrients and also toxic substances from soil, water and air.

Considering the results of our ICP-MS analysis *Pelargonium roseum* can be useful source of Mn, Zn and especially Fe. The ICP technique was well suited for the multi-elemental analysis of vegetable samples in order to ascertain the nutritional role of medicinal plants and the daily dietary intake.

Taking in consideration the high content of Fe of certain vegetative parts of *Pelargonium roseum*, and the fact that people's need of Fe is not entirely satisfied through nourishment, *Pelargonium roseum* might be used in order to supplement Fe input.

Another advantage can be the fact that *Pelargonium roseum* is a plant rich in Fe and with reduced tannin content; consequently the biodisponibility of Fe is not impeded. All these aspects are arguments for a possible use of a capsule which contains powder obtained from this plant as a nutritional supplement in case of iron deficiency.

It must, however, be stated that, because plants may absorb elements from the soil and environment, some of which may be toxic to humans, plant nutrition, climate, and soil conditions and locations could also determine the elemental contents.

Our results are a contribution to a better valorization of this medicinal plant. However further studies will be worthwhile to be performed to confirm the safety of these potential products as dietary supplements, to characterize active principles, and assess toxicity by laboratory assays.

References

- Baratta, M.T., Dorman, H.J., Deans, S.G., Figueiredo, A.C., Barroso, J.G., Ruberto, G., 1998. Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour Frag. J.* 13, 235-244.
- Cavar, S., Maksimovic, M., 2012. Antioxidant Activity of Essential Oil and Aqueous Extract of *Pelargonium graveolens* L'Her. *Food Control.* 23, 263-267.
- Dorman, H.J.D., Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* 88, 308-316.
- Dzamic, A.M., Sokovic, M.D., Ristic, M.S., Grujic, S.M., Mileski, K.S., Marin, P.D., 2014. Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. *J. Appl. Pharm. Sci.* 4(3), 1-5.
- Lancu, C.E., Cioanca, O., Mircea, C., Hăncianu, M., 2013. Contributions regarding leaf histo-anatomy of some *Pelargonium* species. *Rev. Med. Chir. Soc. Med. Nat. Iași.* 117(3), 812-818.
- Lis-Balchin, M., Deans, S.G., Hart, S., 1996. Bioactivity of commercial Geranium oil from different sources. *J. Essent. Oil. Res.* 8, 281-290.
- Mahdieh, M., Yazdani, M., Mahdieh, S., 2013. The high potential of *Pelargonium roseum* plant for phytoremediation of heavy metals. *Environ. Monit. Assess.* 185: 7877-7881.
- Mihaylova, V., Lyubomirova, V., Djingova, R., 2013. Optimization of sample preparation and ICP-MS analysis for determination of 60 elements for characterization of the plant ionome. *Intern. J. Environ. Anal. Chem.* 93(3), 1441-1456.
- Merian, E., Anke, M., Ihnat, M., Stoeppeler, M., 2008. Elements and their compounds in the environment: occurrence, analysis and biological relevance, 2nd ed., Wiley VCH Verlag, Weinheim.
- Moyo, M., van Staden, J., 2014. Medicinal properties and conservation of *Pelargonium sidoides* DC. *J. Ethnopharmacol.* 152(2), 243-55.
- World Health Organization. Guidelines for the Assessment of Herbal Medicines WHO/TRM/91.4. Geneva, Switzerland: WHO.1991.
- World Health Organization. Quality Control Methods for Medicinal Plant Materials. Geneva, Switzerland: WHO. 1998.

Резиме

Определување на содржина на минерали во *Pelargonium roseum* со примена на ICP-MS

Кармен Галеа¹, Габриел Ханку^{2*}, Атила Цизер³, Ценгеле Моника Жеженски⁴,
Енико Барабаз⁵

¹ Катедра за фармацевтска хемија, Фармацевтски факултет, Универзитет „Луциан Блага“, Сибиу, Романија

² Катедра за фармацевтска хемија, Фармацевтски факултет, Универзитет за медицина и фармација,
Тиргу Мурес, Романија

³ Национален Институт за Јавно здравје, Тиргу Мурес, Романија

⁴ Медицински факултет, Универзитет за медицина и фармација, Тиргу Мурес, Романија

⁵ Катедра за ботаника и клеточна биологија, Фармацевтски факултет, Универзитет за медицина и фармација,
Тиргу Мурес, Романија

Клучни зборови: *Pelargonium roseum*, анализа на микроелементи, ICP-MS, додатоци во исхрана

Содржината на микроелементите во различни вегетативни органи на *Pelargonium roseum* е испитувана со метод на индуктивно спрегната плазма - масена спектрометрија (ICP-MS). Аналитичкиот метод овозможи определување на 20 елементи (Li, Be, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Se, Rb, Sr, Ag, Cd, In, Ba, Tl, Pb, Bi). Примероците од различни вегетативни органи (корени, лигнифицирани стебла, зелени стебленца, листови, пупки) се анализирали и споредени со примероци на почва. Вегетативните органи на растението содржат значителни количини на манган, цинк и особено железо. Добиените резултати можат да бидат мошне корисни за определување на препорачана доза на делови од растението (растителни органи) за употреба во медицински растителни производи со оглед на неговата нутритивна вредност и фармаколошките функции.