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Elemental characterisation of the medical plant *Alchemilla velebitica*

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ABSTRACT

Alchemilla, commonly called "lady's mantle", is a genus of herbaceous perennial plants belonging to the family Rosaceae. The species *Alchemilla velebitica* is found only in Southern Europe, like in the Croatian National Park Northern Velebit. Its benefits, such as a astringent and emmenagogue activity as well as wound healing are correlated to the organic compounds found in the plant, but also certain trace elements are known to reduce skin lesions, such as zinc, selenium, copper, manganese, silicon and lithium.

Thus the objective of the present study was the elemental characterization of leaves, blossoms and roots of *A. velebitica*. After acidic microwave assisted digestion the concentrations of selected essential and trace elements were determined by inductively coupled plasma – atomic emission spectrometry and inductively coupled plasma – mass spectrometry. Other minor elements, such as Al, B, Cu, Fe, Mn, Sr and Zn are also found in leaves, blossoms and roots with contents in μg/kg range. The preparation of decoctions and the extraction yields of the elements of interest are calculated.

Introduction

The medical herb *Alchemilla velebitica* commonly called "lady's mantle" belongs to the family Rosaceae and is found only in Southern Europe, like in the Croatian National Park Northern Velebit [1]. There are about 300 species distributed mainly in Europe and Asia. Due to its astringent and emmenagogue activity it can be used to stop internal as well as external bleeding and in cases of diarrhea and gastroenteritis. Furthermore it reduces women related symptoms such as heavy menstrual bleeding, menstrual cramps and menopausal problems. As decoction or wash it can be also applied for treating wounds and skin irritation. These benefits are correlated to the organic compounds found in the plant, but also certain trace elements are known to reduce skin lesions, such as Zn, Se, Cu, Mn, Si and Li [2]. Several studies describe flavonoids as characteristic constituents of European collections of *Alchemilla* [3,4], but there is no report on the elemental content of *Alchemilla* herbs including endemic *A. velebitica.*

Trace elements play a very important role in the formation of the active chemical constituents present in medicinal plants and they are, therefore, responsible for their medicinal as well as toxic properties [5,6]. Important "trace" or minor dietary elements are necessary essential nutrients for humans (zinc, iron, copper, chromium, molybdenum, selenium and cobalt) and only become harmful at high concentrations, while others (lead and cadmium) can present a health risk [7–11].

For assessing the levels of trace and major elements in medical plants, fast and simultaneous methods are applied. Most described in research literature are ICP-AES and ICP-MS, whereby the former is used for elements present in higher concentrations, such as Ca, Fe, and Mg [12]. The latter can avoid results below LOD for trace elements, like Cd and Pb [13].

Aim of the present work is the determination of the element composition in *A. velebitica* from Croatia. After acidic microwave assisted digestion the concentrations of elements were determined by inductively coupled plasma – optical emission spectrometry and inductively coupled plasma – mass spectrometry. The preparation of infusions used for medical purposes and the extraction yields of the elements of interest are calculated.

Materials and methods

Chemicals and glasswares

For the experimental work nitric acid (65 wt% p.a.), hydrogen peroxide (30 wt% p.a.) and p.a. single element standards (ICP

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Multi-element Standard IV, Merck, Darmstadt, Germany) were used. The standard reference material of strawberry leaves (LGC7162) was purchased from LGC Standards (Middlesex, United Kingdom), and TM 27.2 from (National Water Research Institute, Ontario, Canada).

For both ICP-AES and ICP-MS, measurements were accomplished by calibration using aqueous mixed standards prepared in HNO_3 (1 mol/L). All calibration curves were based on five standards, including a blank. Standard stock solutions (1 g/L) were used. For the preparation of aqueous standard solutions, appropriate dilutions of a 1 g/L single element or multielement solution were applied. The calibration ranges were selected according to the expected concentrations of the elements of interest and depending on the technique applied (ICP-AES and ICP-MS).

All glasswares were cleaned with nitric acid prior to use. Ultrapure water produced in-house was used for all dilutions and blanks.

Collection and preparation of sample

A. velebitica herb samples (leaves, blossoms and roots) were collected in July 2012 from Croatian National Park Northern Velebit (N 44,46°–E 14,59°; A 1400 m). All plant parts were washed with distilled water, dried and stored in paper bags at room temperature prior to analysis. The samples were homogenized in a metal free mortar.

For the microwave assisted digestion of the medical herb samples a MWS-2 Microwave System Speedware BERGHOF was used, and the slitly modified digestion procedure described previously [14]. Approximately 0.15 g of the medical herb part (leaves, blossoms and roots) was weighed into a Teflon reaction vessel (in triplicate). The samples were digested with 4 mL HNO_3 (50:50, v/v) + 2.0 mL H_2O_2 according to the following optimized program (1. −150 °C/15 min, 2. −175 °C/15 min and 3. −130 °C/15 min) and measured by both ICP-AES and ICP-MS. Additionally, a standard reference material of strawberry leaves (LGC7162) was digested and analyzed by both methods.

To prepare tea or 'infusion', dried plant leaves and blossoms (approx. 1 g) were added to 98 mL boiling water for different time periods (1, 5, 10, and 20 min). After cooling, the extract was filtered through filter paper (Whatman No. 541).

ICP-AES measurements

The mineral content of the digests and the infusions was determined with a Prodigy High Dispersive ICP-AES spectrometer (Teledyne Leeman, Hudson, NH, USA) working in a simultaneous mode. The spectrometer is equipped with a high resolution Echelle polychromator and a large format programmable array detector (L-PAD), RF-Generator (40 MHz "free-running", output power 1.1 kW), a peristaltic pump (sample uptake flow 1.0 mL/min), a pneumatic nebulizer, and a glass cyclonic spray chamber. Measurements were run with optimal argon flows (coolant: 18 L/min, auxiliary: 0.8 L/min, axial plasma viewing, a sample uptake delay of 30 s and 3 replicates. The emission lines selected for the determination of the elements (wavelength in nm) were: Al (396.152), B (249.677), Ba (455.403), Ca (396.847), Cd (214.441), Co (228.615), Cr (267.716), Cu (324.754), Fe (259.940), K (769.897), Mg (285.213), Mn (259.372), Mo (202.030), Na (589.592), Ni (231.604), Pb (220.353), Sr (407.771), and Zn (213.856). The limits of detection (LOD) were calculated according to Boumans using 3σ and limits of quantification (LOQ) using 9σ for pure element standards and microwave digested samples by measuring an appropriate reagent blank solution ten times and spiked microwave assisted digested medical herb samples also ten times.

The accuracy of the method was determined by analysing a standard reference material of strawberry leaves (LGC7162) using HNO_3 and H_2O_2 for digestion. For the elements not contained in the CRM the recoveries of the analytes were measured at different concentrations using spiked solutions and used as estimation of the accuracy of the method. Spiking experiments were carried out at two concentration levels (1.0 and 5.0 mg L^{-1}) by adding aqueous multielement standard solutions to a set of samples prepared as described above. All spiked samples were prepared in triplicate and measured by ICP-AES. Amount found divided by expected amount multiplied by 100 gives the recovery in percent.

The precision was evaluated by measuring the repeatability of the method for all analytes and kinds of samples. The sensitivity of the method with respect to each metal was evaluated using the resulted slope of the calibration curves. Since good repeatability is obtained even without internal standard, only external calibration was used for quantification. All samples and blank solutions were measured in triplicate.

ICP-MS measurements

Elemental analysis was carried out on an Element 2 ICP-SFMS (Thermo Fisher, Bremen, Germany) equipped with the self aspirating PFA microflow nebulizer (ESI) at a flow of 100 mL/min a PC^3 cyclonic quartz chamber (ESI) operated at 4 °C, a quartz injector pipe and torch (Thermo Fisher), aluminum sampler and skimmer cone (Thermo Fisher). The following operating conditions were applied: RF power of 1300 W and plasma gas flow of 16 L/min, sample gas and auxiliary gas flows were set to 1.06 L/min and 0.86 L/min, respectively.

The isotopes analyzed were at low resolution ^{7}Li, ^{82}Se, ^{88}Sr, ^{111}Cd, ^{208}Pb; at medium resolution ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn and ^{98}Mo and at high resolution ^{75}As. ^{115}Indium (1.1 μg/L) was used as internal standard at all resolutions. Nominal mass resolutions of the Element 2 ICP-SFMS for low resolution (LR), medium resolution (MR) and high resolution (HR) are 350, 4500 and 10,000, respectively.

Merck VI multi-elemental standard was diluted for the external calibrations with 2% HNO_3. For calibration quality control TM 27.2 (Certified Reference Waters for Trace Elements) was used, whereby the values registered agreed with the certified ones within measurement uncertainty. All samples and blank solutions were measured in triplicate.

Results and discussion

Herbal parts

For microwave digested samples (leaves, blossoms and roots) the limits of detection (LOD in mg/kg dried plant part) were: Al (0.0822), B (0.0509), Ba (0.0419), Cd (0.0351), Co (0.073), Cr (0.142), Cu (0.169), Fe (0.119), K (0.0147), Mg (0.488), Mn (0.0099), Na (0.430), Ni (0.127), Sr (0.0105) and Zn (0.116) and higher values for Mo (1.68), Pb (1.57) and Ca (3.00).

Consequently, the detection power of the proposed ICP-AES method is considered satisfactory for routine analysis and quality control. In order to assess the accuracy of a method, standard reference SRM materials are analyzed and the obtained results were compared with the certified ones leading to recoveries in the range from 92 to 105%. Also, the accuracy of the ICP-AES method was estimated by determining the recoveries of the analytes by spiking experiments. For all elements after microwave assisted digestion of *A. velebitica* leaves the recoveries range from 89 to 124% and the relative standard deviation range from 0.43 to 5.6%, proved this method was accurate and precise. For microwave digested samples (leaves, blossoms and roots) the recoveries (in %) were: Al (108.7), B (109.1), Ba (89.2), Ca (109.2), Cd (117.9), Co (116.6), Cr (117.9), Cu