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Investigation of microelements contents in aerial parts of *Agrimonia eupatoria* L., collected in Lviv region (Ukraine)

Introduction

Medicinal plants have a long history of use in therapy throughout the world and still play an important role in traditional medicine. The use of herbal products as the first choice in self-treatment of minor conditions continues to expand rapidly across the world. This makes the safety of herbal products an important public health issue. Thus, medicinal plants and herbal products must be safe for the patient (consumer) (Kosalec et al., 2009).

Man-made environmental pollution can largely affect heavy metal contamination levels of herbal materials. It includes emissions from factories, leaded petrol, agrochemicals such as cadmium-containing fertilizers, organic mercury and arsenic-based pesticides that are still in use in some countries (*WHO Guidelines for…*, 2007). Herbal products can be contaminated at any stage of production, from growing conditions to open-air drying, preserving, and manufacturing (e.g. release from lead-containing utensils). Harmful contaminants may also originate from the conditions in which the medicinal plants are cultivated, post-harvest treatment of herbal material (e.g. fumigants), and finished product manufacturing stages (e.g. organic solvent residues) (Chan, 2003; Kosalec et al., 2009).

The genus *Agrimonia* L. of the family Rosaceae Juss. is known to include valuable medicinal plants. In particular, *Agrimonia eupatoria* L. is indigenous to Middle and Northern Europe, temperate regions of Asia and North America (Gruenwald et al., 2000). The material of commerce is usually imported from Bulgaria, Hungary and Croatia (Wichtl, 2004).

Polyphenols are major constituents of *A. eupatoria* and exemplified by phenolic acids, flavonoids, ellagitannins, and procyanidins. Among flavonoids, two groups of compounds were characterised: the first group consists of flavanols described as quercetin and kaempferol derivatives, and the second one are flavones containing luteolin or apigenin as aglycone moiety. Agrimoniin is reported as a major ellagitannin occurring in common agrimony in high quantities (Granica et al., 2015).

The major compounds in its aqueous-alcoholic extract are flavonoids (0.33%), great amounts of tannins (10.08%), as well as phenolic acids (2.26%), including luteolin 7-O-glucoside and apigenin 7-O-glucoside, luteolin 7-O-sophoroside, luteolin 7-O (6"- acetylglucoside), acacetin 7-O-glucoside; protocatechuic, vanillic, and *p*-hydroxybenzoic acids (Shabana et al., 2003); apigenin, luteolin, kaempferol (Wichtl, 2004; Barnes et al., 2007), quercetin, quercitrin, and glycosides (Barnes et al., 2007).

The monograph for agrimony herb is included into the British Herbal Pharmacopoeia, British and European Pharmacopoeias, Complete German Commission E, Martindale (35th edition) (Barnes et al., 2007). A standardised medicinal plant material (*European Pharmacopoeia…*, 2013), consisting of dried flowering tops of *A. eupatoria* L., contains minimum 2.0% of tannins, expressed as pyrogallol (dried drug); besides of macroscopic and microscopic authentication, for its quality control thin layer chromatography is used (identification C), applying isoquercitroside and rutin as reference solutions, with further UV detection at 365 nm, spraying with solution of diphenylboric acid aminoethyl ester in methanol.

Kurkina et al. (2013) recommend to use cynaroside (luteolin-7-O-glucoside) as a standard sample for purposes of the standardisation of *A. eupatoria* herb.

Agrimony is well known for its beneficial effects in various diseases such as liver complaints, gall-bladder stones; diarrhea, edemas and kidney problems. Approved by Commission E common applications comprise diarrhea, inflammation of the skin, inflammation of the mouth and pharynx. This species is used internally for mild, non-specific, acute diarrhea, inflammation of kidney and bladder, diabetes and childhood bedwetting, cholestasis, inflammation of oral and pharyngeal mucosa; externally for poorly healing wounds, chronic pharyngitis, psoriasis, seborrhoeic eczema, as well as in hip-baths for lower abdominal conditions. It is applied in Chinese medicine as a hemostyptic. It is also used for certain forms of cancer and as an anthelmintic (Gruenwald et al., 2000).

Agrimony is stated to possess mild astringent and diuretic properties. It has been used for mucous colitis, grumbling appendicitis, urinary incontinence, cystitis and as a gargle for acute sore throat and chronic nasopharyngeal catarrh (Barnes et al., 2007).

In experimental model of type 1 diabetes antihyperglycaemic, insulin-releasing and insulin-like activities of agrimony's aqueous extract, given orally to mice, have been demonstrated (Swanston-Flatt et al., 1990; Gray, Flatt, 1998). The herb is shown to be a perspective therapeutic, especially for treatment of social significant diseases such as diabetes and obesity, accompanied by low-grade inflammation. Accumulated evidence suggests that antioxidant activity of *A. eupatoria* might be due to chemical structure of polyphenols and/or herb ability to activate the endogenous antioxidant defence systems (Ivanova et al., 2011). Due to antioxidant properties of flavonoids the herb has shown to exert neuroprotective effects (Lee et al., 2010).

The ethanolic-aqueous extracts of *Herba Agrimoniae* showed the immunostimulant activity in inbred mice resulting in a rise of phagocytic activity and phagocytic index of peritoneal macrophages, in a bactericide action of disintegrated peritoneal cells on *Escherichia coli* cells and in an increased lysozyme activity of supernatants of disintegrated peritoneal cells. The extract of *Herba Agrimoniae* stimulated peritoneal cells to the increased peroxidase activity (Bukovsky, Blanarik, 1994).

Significant uricolytic activity has been documented for agrimony infusions and decoctions (15% w/v), following their oral administration to male rats at a dose of 20 mL/kg body weight (equivalent to 3 g as a dry drug). Diuretic activity was stated to be minimal and elimination of urea unchanged (Giachetti et al., 1986; Barnes et al., 2007). Finally, marked antibacterial activity against *Staphylococcus aureus* and α-haemolytic streptococci has been reported for agrimony (Petkov, 1986).

Therefore, the aforementioned data concerning valuable pharmacological effects of *A. eupatoria* encourage all-side investigation of the medicinal plant since its application in the phytopharmaceutical practice is still underestimated. With that purpose in mind, we have summarized current scientific data concerning its biological activity, paying a special attention to those, which might be useful for renal impact, and also carried out analysis of its trace elements.

Materials and methods

Trace elements in the investigated plant material were quantitatively determined by atomic absorption spectrometry with electrothermal atomization (AAS/EA) with a Zeeman background correction after total microwave – assisted digestion (mineralization) of samples by means of their conversion into soluble forms and further determination for concentration in the solutions (a method of standard additions) in sealed analytical autoclaves.

Reagents

All the reagents were of analytical grade; highly purified water was obtained in accordance with the requirements of the *European Pharmacopoeia* (2013). As a matrix modifier for electrothermal atomisation palladium was used (10 g/dm^3). The blank solutions of manganese, copper, selenium, lead and cadmium with the certified value of the mass ion concentration of 1.0 mg/cm^3 were applied. Reference solutions of the specified elements were prepared by a step dilution of the blank with 1% nitric acid solution from 1 mg/cm^3 concentration to 10 μg/cm^3 (solution

A), followed the obtained solution A by treatment with the same reagent to A concentration of 0.1 μg/cm³.

To construct a calibration graph reference solutions of different concentrations of the trace elements were used (within the operating range for determination of the spectrophotometer), obtained from the blank solutions (0.1 g/cm³) by the standard addition method (*European Pharmacopoeia*…, 2013).

Preparation of the samples

The procedure for a sample preparation was based on a total mineralization technique with a mixture of nitric acid and highly purified water in the reaction chamber of Teflon analytical autoclave with microwave decomposition of the samples under conditions of high pressure.

Accurately weighted samples (0.6 g) of the subjected plant materials, placed in a Teflon vessel, were introduced directly into the reaction chamber of an autoclave, adding 3 ml of highly purified water, followed by addition of 6 ml of 65% nitric acid. The closed autoclave was heated in a microwave digestion apparatus "Milestone Start D". Programme of temperature changes in autoclaves during the period of mineralization is presented in table 1.

Tab. 1. Parameters of temperature changes over time of mineralization in autoclaves

Stage	Time [min. sec.]	Temperature [°C]	Radiation power [W]
1	5.00	80	≤350
2	3.30	160	≤800
3	4.30	195	≤1000
4	14.00	195	≤800

The resulting test solution was cooled after mineralization and quantitatively transferred from the reaction chamber of the autoclave into a 25 cm³ volumetric flask, diluting its contents to volume with highly purified water.

The solutions of the samples were analyzed on atomic absorption spectrometer (VARIAN AA 240 Zeeman Atomic Absorption Spectrometer), electrothermal atomiser equipped with a graphite cuvette (GTA 120 Zeeman Graphite Tube Atomizer) and autosampler (PSD 120 Programable Sample Dispenser). The physical and technological parameters, followed in determining the quantitative content of the microelements were presented (Tab. 2).

The plant material (*Herba Agrimoniae*) consisting of dried aboveground parts of *A. eupatoria* was collected at flowering period (June, 2016) in the suburbs of Lviv, western part of Ukraine and identified by the authors.

Tab. 2. Parameters of atomic absorption spectrometry for analysis of trace elements in plant materials

Trace element	Wavelength of absorption (resonance) lines [nm]	Voltage of resonance radiation lamp [mA]	Flow rate of argon [L/min]	Ashing temperature [°C]	Atomization temperature [°C]	Slit width, [nm]
Manganese	279.50	10	0.30	700	2400	0.20
Copper	327.40	10	0.30	800	2300	0.50
Selenium	196.00	10	0.30	1000	2600	1.00
Lead	283.30	5	0.30	400	2100	0.50
Cadmium	228.80	5	0.30	250	1800	0.50

A composite sample weighing about 10 g consisted of about five subsamples taken within an area of about 10 m^2. All samples were placed in polyethylene bags and transported to the laboratory on the day of sampling (Gałuszka et al., 2015).

Results and discussion

Chronic kidney disease is associated with low concentration of serum selenium and lower platelet glutathione peroxidase (GPx) activity (Kuo, Tarng, 2010). Ceballos-Picot et al. (1996) demonstrated lower serum levels of glutathione and plasma GPx activity in renal failure patients. Plasma selenium is reduced in dialysis patients; there is also significant deficiency and downregulation of glutathione peroxidase, copper-zinc superoxide dismutase, and manganese superoxide dismutase in renal dysfunction (Kuo, Tarng, 2010).

In laboratory animals, parenteral administration of organic and inorganic selenium (210 to 12 000 μg/kg) has been shown to protect against cisplatin-induced nephrotoxicity. The glutathione (GSH) peroxidases are the best-characterized selenoproteins, although other circulating selenoproteins also have antioxidant functions (Ebadi, 2007).

Antal et al. (2010) consider *Agrimonia eupatoria* herb as the plant material able to accumulate higher Se amounts, when this element is present in adequate amounts in the soil.

Cynaroside (luteolin-7-O-β-D-glucoside), another significant substance of agrimony herb, was comparable to the well-known medicinal preparation Lespenephryl with respect to hypoazotemic action. A technology has been developed for producing a medicinal form of cynaroside in 0.05 g tablets (Mamatkhanova et al., 2009).

Many contaminants occur naturally in the ground and the atmosphere, such as radionuclides and metals. Some arise from past or present use of agents that pollute the environment and subsequently medicinal plants, such as factory emissions or persistent chemical residues. Due to their excessive use and disposal, contaminants from environmental sources may even be present if a herb is organically grown. Metals are widely distributed throughout nature and occur freely in soil and water. As they are likely to be present in many food sources, it is important to reduce the total population exposure to toxic elements by minimizing contamination of herbal products (*WHO Guidelines for…*, 2007; Kosalec et al., 2009).

Agrimony is listed by the Council of Europe as a natural source of food flavouring (category N2). This category indicates that agrimony can be added to foodstuffs in small quantities, with a possible limitation of an active principle (as yet unspecified) in the final product (Barnes et al., 2007).

Scientific data concerning contents of trace elements of agrimony herb are rather limited and are related to several publications (Štroffeková et al., 2006, 2008; Antal et al., 2010; Gałuszka et al., 2015), where the samples of *Herba Agrimoniae* from Slovakia, Romania and Poland were analysed. Therefore, it seems important to investigate contents of trace elements in the herbal drug, collected in wild in the Western Ukraine, for comparison of the found outcomes with the known ones related to the subjected medicinal plant material.

The content of the elements in above-ground portions of *A. eupatoria*, collected in two different Slovak regions, determined by the method of radionuclide X-ray fluorescence (source ^{238}Pu) was the following: Mn – 36.81 μg/g, Cu – 7.70 μg/g, Pb – 2.26 μg/g (Nové Mesto Nad Váhom) and Mn – 25.07 μg/g, Cu – 7.53 μg/g, Pb – 2.51 μg/g (Ružomberok) (Štroffeková et al., 2006).

Metals contents for agrimony herb samples, grown in non-chemical treated meadows in Nitra (Slovakia), performed using X-ray fluorescence analysis (XRF) (source ^{238}Pu for lead, and ^{241}Am for cadmium) and galvanostatic stripping chronopotentiometric analysis (SCP) were determined as follows: Pb – ≤ 1.95 μg/g, Cd – ≤ 1.96 μg/g (XRF) and Pb – 1.043 μg/g, Cd – 0.279 μg/g, Se – 0.175 μg/g (SCP). XRF is an official method in *European Pharmacopoeia* (2013), it is described as X-ray spectrometry (Štroffeková et al., 2008).

For the plant samples, collected from limestone soils in two localities of the Aninei Mountains (Banat region, Romania), believed to be unpolluted (far away from roads, kilometers outside villages or towns), outstanding Se contents (332 μg/kg) was measured for *A. eupatoria* herb from Lisvar Hill; the samples from Ciopliaia Hill yielded 61 μg/kg of selenium (Antal et al., 2010).

A semiquantitative multi-element analysis by inductively coupled plasma mass spectrometry (ICP-MS) of aboveground parts of agrimony, collected in a residential area of the city of Kielce, (Góry Świętokrzyskie), south-central Poland, allowed determining the following contents of the trace elements in the subjected plant material: Mn – 44 mg/kg, Cu – 8.0 mg/kg, Pb – 0.001 mg/kg, Cd – 0.001 mg/kg (Gałuszka et al., 2015).

The found quantities of trace elements in the analyzed by us samples of *Herba Agrimoniae*, collected from wild in the suburbs of Lviv, are as follows: Mn – 46.9 μg/g, Cu – 7.9 μg/g, Se – 0.16 μg/g, Pb – 0.15 μg/g, Cd – 0.05 μg/g.

Comparing the received outcomes with the other known investigations, it might be concluded that the contents range of the trace elements in *Herba Agrimoniae*, collected from various locations in Europe, comprise for Mn – 25.07–46.9 μg/g; Cu – 7.53–8.0 μg/g; Se – 0.061–0.332 μg/g; Pb – 0.15–2.51 μg/g; Cd – 0.05–0.279 μg/g.

The WHO recommends the following limits for heavy metals in herbal drugs: 10 mg/kg for lead and 0.3 mg/kg for cadmium (*Quality Control Methods…*, 1998; *WHO Guidelines for…*, 2007). Therefore, the analysed by us herbal drug complies with the requirements of the WHO concerning the contents of lead and cadmium.

Conclusions and recommendations

With an objective to determine quality characters, as well as contents of 5 trace elements (manganese, copper, selenium, lead, cadmium) in the aboveground parts of agrimony, official herbal drug, grown wild in the western part of Ukraine, there has been carried out an atomic absorption spectroscopy with electrothermal atomization after mineralization. The received research outcomes were compared with results of other known scientific investigations of the subjected medicinal plant material.

The determined yield of microelements allows recommending the herbal drug for further biological investigations, as well as a promising nephroprotective medicine.

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Abstract

The genus *Agrimonia* L. of the family Rosaceae Juss. is known to include valuable medicinal plants. Agrimony is well known for its beneficial effects in various diseases such as liver complaints, ga\ll-bladder stones; diarrhea, edemas and kidney ailments. The contents of microelements in aerial parts of *Agrimonia eupatoria*, an official herbal drug, collected from wild samples in the suburbs of Lviv city, Western Ukraine, have been determined by atomic absorption spectroscopy after mineralisation in the microwave Milestone Start D. The found quantities of trace elements in the analysed samples were as follows: manganese – 46.9 μg/g, copper – 7.9 μg/g, selenium – 0.16 μg/g, lead – 0.15 μg/g, cadmium – 0.05 μg/g The received research outcomes were compared with results of other known scientific investigations of the medicinal plant material. The determined amounts of lead and cadmium in the investigated samples of the herbal drug complied with the World Health Organization (WHO) requirements. The special attention is paid to pharmacological effects, related to its renal impact, contents of selenium and phenolic compounds that allow considering the herbal drug as a promising nephroprotective agent.

Key words: AAC/EA (atomic absorption spectroscopy with electrothermal atomization), *Agrimonia eupatoria*, microelements, renoprotective, selenium, trace elements

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Badanie zawartości mikroelementów w nadziemnych częściach *Agrimonia eupatoria* L., zebranych w obwodzie lwowskim (Ukraina)

Streszczenie

Rzepik pospolity *Agrimonia eupatoria* L. z rodziny Rosaceae Juss. należy do cennych roślin leczniczych. Jest dobrze znany ze swoich korzystnych działań w różnych chorobach, takich jak: dolegliwości wątroby, kamienie pęcherzyka żółciowego, biegunka, obrzęki i dolegliwości nerek. Próbki do badań z tego oficjalnego leku ziołowego pozyskano ze stanu naturalnego w okolicach Lwowa (Zachodnia Ukraina). Zawartość mikroelementów w częściach nadziemnych *A. eupatoria* zbadano metodą atomowej spektroskopii absorpcyjnej, po mineralizacji mikrofalowej w Milestone Start D. W analizowanych próbkach znaleziono śladowe ilości pierwiastków, takich jak: mangan – 46,9 ug/g, miedź – 7,9 ug/g, selen – 0,16 ug/g, ołów – 0,15 ug/g, kadm – 0,05 ug/g. Uzyskane wyniki badań porównano z innymi znanymi z literatury badaniami na materiale roślinnym z tego gatunku. Stwierdzone w badanych próbkach ilości ołowiu i kadmu są zgodne z wymogami Światowej Organizacji Zdrowia (WHO). Szczególną uwagę zwrócono na efekty farmakologiczne tego substratu, związane z jego wpływem na nerki, zawartością selenu i związków fenolowych, które pozwalają uznać ten lek za obiecujący środek chroniący nerki.

Słowa kluczowe: AAC/EA (spektroskopia absorpcji atomowej z atomizacją elektrotermiczną), *Agrimonia eupatoria*, mikroelementy, nefroprotekcja, pierwiastki śladowe, selen

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He carries out a focused analysis of plant species for selenium contents and the marker substances of flavonoid structure, that cause nephroprotective (hypoazotemic) activity, and investigates renal impact of their extracts. Research interests also comprise principles and improving of teaching of Pharmacognosy for English-medium students. Author of over 55 scientific publications, including 3 manuals, 1 electronic textbook, 5 educational and methodical recommendations.

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