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Cent. Eur. J. Chem. • 8(2) • 2010 • 264–268
DOI: 10.2478/s11532-009-0133-9

Central European Journal of **Chemistry**

A preliminary study on the effect of mineralization parameters on determination of metals in *Viscum album* species

Short Communication

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Received 15 June 2009; Accepted 26 September 2009

Abstract: An increasing interest in determination of various macro- and microelements in medicinal plants has been observed. The majority of studies are carried out using one mineralization method without any optimization. The present study demonstrates that changes in mineralization parameters can significantly affect the recovery of the elements determined. In the study, the dried plant material was mineralized in 12 ways and iron (Fe), copper (Cu), zinc (Zn), nickel (Ni) and manganese (Mn) levels were determined. The samples were mineralized in the dry or open microwave mode as well as 10 closed microwave modes. The influence of acid amounts, irradiation power and time, addition of hydrogen peroxide and perfluoric acid was examined. All parameters were shown to be critical - good efficiency was observed with larger amounts of acid. The determined content varied significantly in the same sample and were in the ranges (ug g^{-1}): 46 – 136 (Fe), 1.4 – 11.8 (Cu), 4.0 – 11.3 (Ni), 15.4 – 53.8 (Zn) and 9.5 – 67.6 (Mn). Increased irradiation resulted in the loss of copper and zinc and better recovery of nickel. The results demonstrate that such determinations should include the mineralization optimization step.

Keywords: *Medicinal plants • Heavy metals • Mineralization parameters • Ion chromatography • Natural drugs*

1. Introduction

Medicinal plants are considered as plants which have potential medicinal properties. They are grown or collected from natural sources, then consumed in various forms as medicines. In recent years, there is an increasing interest in their use all over the world, as an alternative to traditional therapy. Different parts of plants are used as spices, herbs, food dressing and additives or herbal teas. Although the efficiency of herbal therapy can be as high as that of chemical drugs, the safety of their use has often been questioned due to the presence of high unacceptable levels of heavy metals [1-5].

The environmental pollution and contamination with toxic heavy metals has been considered a significant problem. The main sources are industrial and traffic emissions, use of purification mud and metal-containing agricultural products [6], which increase heavy metal concentrations in soil, resulting in elevated levels of these metals in food and herbs. The heavy metal levels in plants depend on the geochemical characteristics of the soil and the ability of plants to accumulate them selectively [7]. Moreover, the content of non-toxic microelements is also important because their content may have significant influence on their therapeutic action [8].

Therefore, the scientific interest in the heavy metal content in medicinal plants is topical. Numerous studies concerning heavy metal levels in medicinal plants were carried out in Algeria [9], Austria [10,11], Bulgaria [12], China [13-17], Malaysia [18], Nigeria [19], Poland [20-22], Serbia [23] and Turkey [24,25].

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The metal content in herbal material is mainly analysed using atomic absorption spectroscopy (AAS) or ion chromatography. The crucial part of sample preparation is the mineralization process, in which the sample is converted from an organic to inorganic form.

The simplest and oldest method of mineralization is a dry process — a sample is burned into ash and the remaining residue is dissolved in acid. The simplicity is the only advantage of this method. The drawbacks include the loss of volatile metals and long duration. The sample can also be mineralized in the oxygen plasma or microwave oven, yet this method requires additional expensive equipment.

Wet mineralization consists of boiling the sample with some very strong oxidizing reagent, such as concentrated acids in an open, closed or flow system. The sample is heated in the classical way by flame, microwave radiation or UV radiation. Although the loss of the volatile metals is very low, there are additional faults: the temperature is relatively low (limited by boiling point of the media) and the sample can be contaminated from the air (open systems). In closed systems, on the other hand, the mineralization process cannot be observed.

Recently, the mineralization optimization has been strongly emphasized. The mineralization process should be optimized to achieve full performance and minimize the loss of volatile content. In the studies mentioned above, only a single mineralization method was applied, whereas such process should be optimized to achieve maximum performance (full mineralization) and minimize the loss of volatile content. Kalembkiewicz *et al*. [26] studied the mineralization optimization during the determination of soil samples. Rejnek [27] compared the dry and wet method during analysis of apple tree leaves and human hair samples, whereas Vidal *et al*. [28] compared acid extraction, wet digestion (a microwave oven) and dry ashing mineralization in food analysis.

The above studies suggest that changes in mineralization parameters can significantly affect the final results. No such investigations have been performed yet on medicinal plants. Therefore, the aim of the present study was to compare the effects of several mineralization parameters on heavy metal levels determined in medicinal plants and construct a preliminary conclusions.

2. Experimental Procedure

The dried plant material of *Viscum album* L. was from Herbapol S.A., Lublin, Poland (purchased in a local drugstore). Six packages from one serie were grounded in the laboratory homogenizer (Testchem, Pszow, Poland) and mixed carefully. The mineralization modes are shown in Table 1. The wet modes (A — J) were carried out in the microwave mineralizer UniClever BM-1z (Plazmotronika, Warsaw, Poland). Accurately weighed samples (0.5 g ± 0.01 g) were placed in a teflon couvette with appropriate mixture of oxidation reagents. After careful mixing, mineralization was conducted in three modes — 17 - 20 atm (gradient pressure) pressure with 60% (relative to full 1050 W) microwave power (Step 1), 27 - 30 atm pressure with 80% power (Step 2) and 42 - 25 atm pressure with 100% power (Step 3), each step of various duration. In mode G, the powers were 40%, 60% and 80%, respectively. After the process, the samples were transferred into 25-mL volumetric flasks and made up to appropriate volume with deionised water.

Table 1. The mineralization parameters used in the investigation.

Procedure	HNO_3 [mL]	H_2O [mL]	H_2O_2 [mL]	HF [mL]	Mode 1 [min]	Mode 2 [min]	Mode 3 [min]
A	3	7	0	0	1	5	6
B	2	8	0	0	1	5	6
C	1	9	0	0	1	5	6
D	3	7	0	0	1	3	5
E	2	8	0	0	1	3	5
F	1	9	0	0	1	3	5
G*	3	7	0	0	1	5	6
H	3	6	1	0	1	5	6
I	2	7	1	0	1	5	6
J	3	6.8	0	0.2	1	5	6
K	Classical dry mineralization						
L	Microwave mineralization in open mode						

** — reduced generator power, see text*

Mode K (classical dry mineralization) was carried out as follows. Accurately weighted samples (0.5 g ± 0.01 g) were placed in quartz crucible, buried in the flame and heated at 450ºC in the electric oven. Partially mineralized samples were moistened by nitric acid and heated again. After total decomposition, ashes were dissolved in 5 mL of concentrated Hcl mixed with 5 mL of water, boiled, cooled and made up to 25 mL with water.

The last mode (mode L) was the open mode microwave mineralization. The samples were mixed with 30 mL of 65% H_2SO_4 and mineralized by microwaves (15 min of 10% power followed by 20 min of 15% power). If the sample was not mineralized, the further process was under closed conditions: 2 min at 17 - 20 atm pressure and 60% power and 10 min at 42 - 45 atm pressure with 100% power.

The samples were analyzed using Dionex DC-500 IC ion chromatograph with post-column derivatization on-line and spectrophotometric detection. The dedicated

Table 2. The mean content [μg g^{-1}] of investigated metals in *Viscum album* samples, determined with 12 mineralization modes (A-K)

Mode	Fe	Cu	Ni	Zn	Mn
A	136	6.5	11.1	50.9	67.6
B	128	6.3	11.3	50.8	66.0
C	94	5.8	10.0	47.6	59.6
D	130	11.8	9.7	51.2	61.3
E	116	6.7	8.2	44.3	56.1
F	102	6.2	6.3	42.5	58.3
G	117	5.7	9.2	53.8	68.3
H	96	6.8	8.8	57.2	58.5
I	92	6.4	5.5	54.5	55.0
J	112	5.0	7.1	48.7	52.8
K	46	1.4	4.0	15.4	9.5
L	83	4.4	7.2	35.6	40.3

pre-column (CGP 5A) and column (CS 5A) was used. The aqueous mobile phase contained 35 mol L^{-1} pyridinecarboxylic acid (PDCA), 0.37 mol L^{-1} formic acid, 0.33 mol L^{-1} KOH and 28 mmol L^{-1} K_2SO_4.

The derivatization was carried out using the aqueous solution containing 2.8 mmol L^{-1} 4-(2-pyrridazo) resorcinol (PAR), 0.3 mol L^{-1} $CaCO_3$, 1 mol L^{-1} dimethylaminethanol and 0.5 mol L^{-1} of ammonia.

Twelve independently weighed samples of each mode were analyzed (144 samples in total). Five metals (Fe, Cu, Ni, Mn and Zn) were determined in each sample.

3. Results and Discussion

The mineralization effect on the analysis must be assessed with proper experimental design. We have chosen 12 modes as follows:

1. modes A — C differ in the amount of HNO_3 added to investigate the influence of the acid content on the process.
2. modes D — F are analogous to A — C (also differ in acid amount), yet the times of the microwave irradiation are shorter. Comparison between the two groups checks the presence of the effect of irradiation time.
3. mode G is analogous to mode A (the same acid amount and the same time), but less microwave power is used to test the influence of microwave power reduction.
4. modes H and I are analogous to A and B, however, addition of H_2O_2 was used.
5. mode J is analogous to A, yet with HF added.
6. mode K is designed to test the differences between classical dry mineralization and microwave mineralization.
7. mode L is designed to compare the differences between closed and open mineralization procedure.

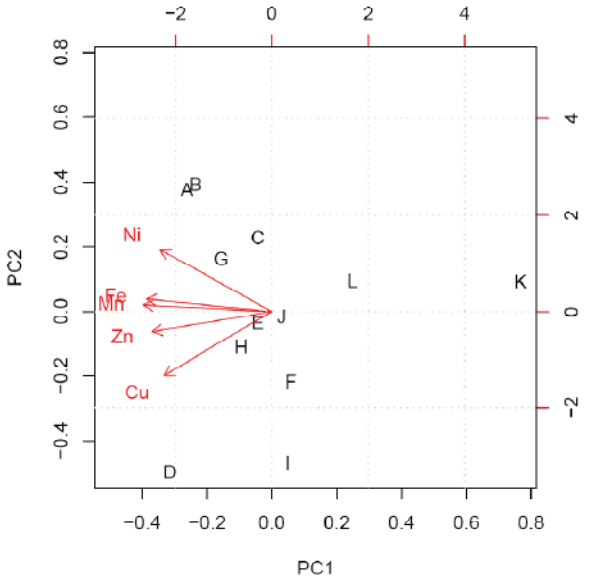

Figure 1. The principal component analysis (PCA) of the results. The two first PCs explain 88.7% of total variance.

Although several dried plant species were tested (*Polygonum avicularis, Equisetum arvense, Humulus lupulus, Frangula annus, Viscum album*), only the *Viscum album* material findings are presented. The results of all other species led to similar conclusions. The mean content (in μg g^{-1}) of the analyzed metals is presented in Table 2. Satisfactory precision (RSD < 6%) was observed in each case.

The analysis of results for *Viscum album* was conducted using scaled principal component analysis (PCA), which decorrelates the variables and converts them into orthogonal linear combinations, *i.e.* principal components (PCs). The initial matrix had 5 columns (metals) and 12 rows (mineralization modes). The first two principal components explain maximum available overall variance, thus their plot can be used for comparison of mineralization methods. The distance between two points on this plot is maximal possible approximation of the Euclidean distance in scaled multivariate space. The results (PCs of methods and corresponding loadings of metal contents denoted as arrows) are presented in Fig. 1.

The first two PCs explain 88.7% of overall variance. The first principal component (with formula PC1 = -0.471Fe – 0.405Cu – 0.421Ni – 0.450Zn – 0.483Mn) denotes the sum of metal content and can be treated as the measure of overall mineralization performance. The negative value of the first PC indicates good recovery of

metal content. It can be noted that the amount of acid added is a significant parameter of the process: the modes A and B are very efficient (strong negative PC1), whereas C performs worse. Mode D is very efficient, whereas E and F give lower recoveries. The classical dry mineralization (K) and open mineralization (L) are the worst choices.

The second PC (calculated as PC2 = 0.136Fe – 0.694Cu + 0.667Ni – 0.218Zn + 0.071Mn) depicts the difference in performance among the metals. It was demonstrated that improving the recovery of nickel worsens the recovery of copper and zinc and *vice versa*. Shorter microwave operation (modes D — F) results in better recovery of copper and zinc, yet is insufficient in the case of nickel. Increased irradiation time (A — F) results in decreased content of zinc and copper, probably due to their volatility.

The effect of the irradiation power reduction (between A and G) is similar to that observed when irradiation time is reduced, although it is not so evident; moreover, the overall performance is also slightly decreased. The addition of H_2O_2 (pairs A — H and B — I) decreases the overall performance and improves the recovery of copper and zinc. The same yet less evident relation is observed in the case of HF addition (pairs A — J).

The copper content in method D should be emphasized, significantly higher than in other methods, which demonstrates that copper is the metal most sensitive to evaporation; thus, reduced irradiation time together with increased acid content, significantly increases its recovery.

4. Conclusions

The mineralization optimization is essential for herbal analysis. The use of one predefined mineralization mode is insufficient; the results are unreliable and their comparison with reference data is not feasible. All the mineralization parameters affect the performance. The plethora of acid is always a prerequisite of good recovery. Long and strong irradiation improves the recovery of nickel, yet worsens the content of copper and zinc. The first preliminary recommendation would be to use two modes: one with long and strong irradiation (good for nickel) and shortest possible (good for copper and zinc). There is no mineralisation mode optimal for all determined metals. Further studies, incorporating more samples and some certified reference materials, are planned by the authors.

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