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Determination of Mineral Elements in *Otostegia Persica* and *Otostegia Aucheri* from Saravan Region, Sistan and Baluchestan, Iran

Sadeghi, Z. [*] **and Valizadeh, J.**

Department of Agricultural Machinery, Shahid bahonar University of Kerman, Kerman, Iran.

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Abstract *Otostegia persica* Bioss and *Otostegai aucheri* L. (Labiatae) are very important for treatment of diabetes mellitus and other various diseases in Saravan region. In this study mineral elements of these plants has been investigated. Two methods of sample preparation, dry ashing and microwave assisted acid digestion, were used for mineralization of these plants. Mineral elements consisting of Ca, Mg, Na, Fe, K, Mn, Cr, Cu, Pb and P were determined by flame and graphite furnace atomic absorption spectrometry. Analysis of mineral elements revealed that these species have high percentage of nutrition, and there was a significant correlation between the dry ashing and microwave digestion methods for mineralization of species ($R = 0.999$, $P < 0.01$). Recovery of Fe in microwave assisted acid digestion was more than dry ashing digestion method. Microwave assisted acid digestion methods for these plants offers considerable advantages, including good safety, speed and reduced contamination. Moreover, it is more favourable and advantageous by supplying more reliable results and being easy to apply for mineral elements determination. As well as this study revealed these plants have high nutritive value that the consumption of theme would terminate several beneficial effects such as antidiabet activity.

Key words: *Otostegia persica*, *Otostegai aucheri*, Mineral elements, Microwave digestion, Dry Ashing

Introduction

The genus *Otostegia* is belonging to Lamiaceae family and include of about 33 species, which grows mainly in the Mediterranean region and adjoining Asia Minor (Khan *et al*., 2009). Three species of this genus are available in Iran: *O. aucheri*, *O. michauxi* and *O. persica*, of which the last two are endemic to Iran (Ghahreman and Attar, 1992, Ayatollahi *et al*., 2009). *O. aucheri* and *O. persica* have widely distributed in Sistan and Baluchestan

[*] **Corresponding author:** Sadeghi, Z.**; E-mail:** sadeghi.phytochem@gmail.com zahra sadeghi <sadeghi.phytochem@gmail.com>

province. Several interesting work of biological and pharmaceutical properties on *O. persica* were published in the last 10 years. This species is traditionally used for treatment of malaria, fever and diabetes

(Sadeghi *et al*., 2014). Additional biological screening of this species has revealed strong antibacterial activities against various Gram-positive strains and Gram-negative bacteria, antimalarial, anti-inflammatory and healing of burn wound, anti-glycation activity as well as anti-aphids and pesticidal activity (Salari *et al*., 2010; Nateghpour *et al*., 2012; Ganjali *et al*., 2013; Ayatollahi *et al*., 2010;Asghari *et al*., 2006). *O. aucheri* has also antidiabetic effects (Sawar *et al*., 2009; Mosihuzzaman, 2005). No further researches have been reported in the literature for mineral element analyses of these species. The determination of the metal content in medicinal plants is important because some of these metals are related to human health. Metal determination in this matrix has traditionally been carried out using wet or dry ashing methods as sample preparation. Wet digestion can be executed with a concentrated acid or mixture of acids in open or closed systems by the use of conventional or microwave heating (Lavilla *et al*., 1999). In this work, two digestion methods carried out microwave assisted acid digestion (wet ashing) and dry ashing. The goal of this work was to develop digestion methods that are safe and fast and use minimum acid volume. From this viewpoint, the levels of Ca, Mg, Na, Fe, K, Mn, Cr, Cu and Pb in *O.persica* and *O.aucheri* were determined by flame and graphite furnace atomic absorption spectrometry after digestion using two methods.

Material and methods

Plant materials

The aerial parts of *O. aucheri* and *O. persica* were collected in the region of Bam Posht in Saravan city during March 2009. Voucher specimen of the plant sample was deposited in the Herbarium (SCH-130 and SCH-131) of department of Production and Utilization of Medicinal Plants, Higher Educational Complex of Saravan.

Analysis of inorganic elements in O. persica and O. aucheri

Two types of digestion procedures were applied for mineralization of plant samples: dry ashing and microwave assisted acid digestions.

Dry ashing

Four gram of sample was weighed accurately and placed into a high form porcelain crucible. Temperature of furnace was slowly increased to 500˚C in 1 hr. The sample was ashed for about 8 hr until a white or grey ash residue was obtained. The residue was dissolved in HNO_3/HCl/H_2O [1:2:3] and heated gently on hot plate until brown fumes disappeared. The solution was transferred to a 100-mL volumetric flask and made up to volume Solution was used for elemental analysis of Ca, K, Mg, Fe, Na, Cu, Cr and Mn by atomic absorption spectrophotometer. Calibration of the instrument was repeated periodically during operation. Mineral were calculated by comparison of their standards solutions. All standards used were of high purity procured from Merck or Sigma. Pb was determined by graphite furnace atomic absorption (Okwu, 2005).

Determination of phosphorus

After dry ashing digestion, Phosphorus content was determined by ammonium molibdate reagent after 10 minutes by UV-Vis in 420 nm. Calibration curves were constructed using four standard solutions for NaH_2PO_4 in the different range.

Microwave assisted acid digestion

0.5gr of crushed air-dried aerial parts of *O.persica* and *O.aucheri* was mixed with 10 mL concentrated HNO_3 in the glassic beaker and was placed inside domestic Microwave oven. Sample was irradiated at a 900 W power for 10 min. After this time, 5 mL of concentrated HCl was added and irradiation was continued for 5 minutes. After digestion, the vessel was cooled, filtered [Whatman No 42 filter paper] and diluted with double distilled water to a final volume of 100 mL (Ravande et al., 2011). Solution was used for elemental analysis of Ca, K, Mg, Fe, Na, Cu, Cr and Mn by atomic absorption spectrophotometer.

Pb was determined by graphite furnace atomic absorption. Quantity of P was determined according to above method.

Statistical analysis

Measurements of mineral elements were done in triplicates to test the reproducibility of them. All results are presented as mean ± S.E. SPSS 15.0 (statistical soft ware) was used for statistical analysis of results. Statistical analyses were performed by Student's t-test. The values of $P < 0.05$ were

considered statistically significant. Correlations among data obtained were calculated using Pearson's correlation coefficient (r).

Results and Discussion

The perecentage of ten elements containing Mg, Ca, Na, K, Cu, Cr, Fe, Pb, Mn and P in were determined in *O.persica* and *O.aucheri* species have been shown in table 1. The study revealed that investigated plants are good source of Na, K, Ca, Mg and Fe. Sample decomposition is one of the most important steps in analytical procedures in biological matrices. This is principally important in the determination of trace elements in plant materials, since they are not homogeneous and usually contain various mineral fractions. The decomposition procedures used for biological materials include dry ashing, wet ashing and direct wet dissolution/microwave digestion. The microwave assisted closed vessel digestion technique is being increasingly used in the digestion of various organic matrices, since this method facilitates rapid dissolution of the sample matrix, requires low oxidising reagent use and causes minimal contamination of the sample prior to the elemental analysis step (Erdogan *et al*., 2006). As results shows recovery of Fe in microwave digestion method was better than dry ashing digestion method. This might be because in dry ashing digestion method, irons form oxides which are difficult to dissolve (Kucak and Blanusa, 1998; Soylak *et al*., 2004). For other elements such as Ca, K, and Mg, similar results were obtained. The concentration of other elements analysed in this study were not different in two methods. The standard deviation was calculated for N = 5 and the value were approximately less than 1%. The elements containing Fe, K, Mg, Na, Ca, Mn and Cu have been classified as essential elements. The highest concentration was related to Ca, Na and K. Calcium is high in O.persica. The first health benefit of calcium is building and maintaining strong bones and teeth. As well as Ca plays important roles in heart and muscle health. It supports nerve transmission and muscle function and can help maintain heart rhythm. Calcium is also necessary for blood coagulation, milk clotting and regulation of cell permeability (Yagi *et al*., 2013). In humans, Mg is required in the plasma and extra cellular fluid, where it helps in preserving osmotic equilibrium. It is a critical co-factor in many enzyme – catalysed reactions. Lack of Mg is associated with abnormal irritability of muscle and convulsions and excess Mg with depression of the central nervous system. Na and K have important roles in ionic balance of the human body and maintain tissue excitability, muscle contraction, gastric juice in stomach. (Intidhar *et al*., 2013), K help in release of chemicals which acts as nerve impulses, regulate heart rhythms, deficiency causes nervous irritability mental disorientation, low blood sugar, insomnia and coma. Na plays an important role

in the transport of metabolites. The ration of K/Na in any food is a serious factor in debarment of hypertension arteriosclerosis, with K depresses and Na increases blood pressure (Bhowmik *et al*., 2012). The amount of Fe in the O.persica in O.aucheri is 0.452 and 0.274 percent respectively, by microwave digestion method. Fe is very important in the haemoglobin structure. It plays a vital role in carry oxygen and electron in human body, normal functioning of the central nervous system and in the oxidation of different biological compound (Nile and Khobragade, 2009). *O.persica* hase high content of Copper. Cu is the third most prevalent mineral in the body and has an important role in many enzyme systems. Cr, Mn and Zn have high value in the cholesterol metabolism as well as heart diseases. The presence of Cr and Mn in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases (Yagi *et al*., 2013; Nile and Khobragade, 2009). Total Pb concentration in these plants was <1000mg kg−1 (Table 1). According to the U.S. Pharmacopoeia, the lead limit for pharmaceutical products is 10 ppm. It is an acceptable limit for plant products, drugs and dietary supplements. Whereas these plants are collected from rural areas. These areas are generally away from industrial, and therefore are free of high levels of lead contamination from the soil and air (Dharmananda, 2001). Pb2+ was found to be toxic and harmful to human beings when present in high amounts. Since Pb2+ is not biodegradable, once soil has become contaminated, it remains a long-term source of Pb^{2+} exposure (Tangahu *et al*., 2011).

The mineral and heavy metal concentration reported here in might not be different compare to some of the other reports on medicinal plants (Nile and Khobragade, 2009; Shahnawaz *et al*., 2012). The differences observed might be due to different growth conditions, genetic factors, geographical variations in the level of soil fertility, efficiency of mineral uptake and the analytical procedure employed (Shahnawaz *et al*., 2012). The data obtained in the present work will be useful in cultivate planting synthesis of new herbal drugs with various combinations of plants, which can be used in the treatment of different diseases at global level generally and in Sistan and Baluchistan, Iran particularly.

Conclusions

Two species of Otostegia were successfully digested by the proposed microwave assisted acid digestion methods. This proposed digestion method for these plants offers considerable advantages, including good safety, speed and reduced contamination. Moreover, microwave assisted acid digestion is more favorable and advantageous by supplying more reliable results, being easy to apply for heavy metal determination. Disadvantages of microwave assisted acid

digestion may be high cost and lack of method experience. Based on this information, it could be concluded that these plants are natural sources of mineral elements. They have high nutritive value that the consumption of these species would terminate several beneficial effects such as antibacterial activity.

Table 1. Percentage of mineral elements [± SD] in O.persica and O.aucheri by two methods of digestion by dry ashing [D.A] and microwave digestion [M.D] [w/w %]

		O.aucheri		O.persica	
No	Elements	D.A method	M.D method	D.A method	M.D method
1	Ca	3.501±0. 12	3.522±0.61	4.136±0.32	4.023±0.24
2	K	2.162±0.57	1.991±0.45	1.161±0.29	1.232±0.16
3	Mg	0.351±0.24	0.341±0.33	0.237±0.73	0.201±0.61
4	Fe	0.015±0.031	0.051±0.028	0.274±0.82	0.452±0.21
5	Na	0.059±0.053	0.043±0.014	0.093±0.035	0.091±0.011
6	Cu	0.002±0.023	0.002±0.069	0.008±0.015	0.007±0.039
7	Cr	0.004±0.018	0.033±0.013	0.026±0.046	0.027±0.055
8	Mn	0.003±0.072	0.004±0.081	0.005±0.091	0.003±0.016
9	Pb	1.532×10^{-5} ±0.0003	1.458×10^{-5} ±0.0002	13.383×10^{-5} ±0.003	12.883×10^{-5} ±0.0006
10	P	0.684±0.66	0.576±0.35	0.706±0.20	0.739±0.43

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