Iran J Sci Technol Trans Sci (2021) 45:737–743
https://doi.org/10.1007/s40995-020-01048-2

RESEARCH PAPER

Elemental Analysis of Two Species of Medicinal Plants Hymenocrater and *Stachys lavandulifolia* by INAA

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Received: 5 July 2020 / Accepted: 5 December 2020 / Published online: 27 February 2021

Abstract

Nowadays, the use of medicinal herbs is very popular and many people use them to treat their diseases, so it is important to know the concentration of trace elements in these herbs. In this study, the concentration of 10 elements was measured by the method of instrumental neutron activation analysis (INAA) in two types of medicinal plants as *Stachys lavandulifolia* and Hymenocrater. Samples were taken from various parts of the cities of Arak and Sarband, as well as from the Alvand zone Hamadan in Iran. This study shows that these plants are rich in magnesium, manganese, zinc, calcium and iron.

Keywords Element · Herbal plant · Neutron activation analysis · Hymenocrater · *Stachys lavandulifolia*

1 Introduction

Today, the number of people using herbal medicine as a supplement or alternative of industrial medicine is increasing. Therefore, it is important to determine the content of useful and harmful or toxic elements contained in them. Many studies have been done on the benefits of herbs, and many articles have been published, including the use of herbs to treat skin diseases, lowering blood cholesterol and blood sugar levels and controlling diabetes (Pourimani et al. 2018; Ke et al. 2012; Kashian et al. 2018). Unlike chemical drugs, medicinal plants contain a large number of active organic substances that are more compatible with the nature of the human body (Mashour et al. 1998). Various methods are used to determine the concentration of elements in medicinal plants, and the method of neutron activation is of particular interest to researchers due to many advantages, such as nondestructive and high accuracy. In this method, using a small amount of material, the concentration of a large number of elements can be determined simultaneously. Many researchers around the world have used this method to study medicinal and edible plants (Monged 2016; Michenaud-Rague et al.2012; Rahimi and Rabani 2010). The main purpose of this article is to identify the elements that are found in Hymenocrater and *Stachys lavandulifolia*. In Iran, these herbs are used to improve the functioning of the human body and are used as drinks containing 50% of each, which are prepared like tea. The *Stachys lavandulifolia* is found mainly in western parts of Iran and grows in mountainous areas. The most important chemical constituents of this plant are hyperin red substance hypericin, tannin and nicotinic acid. This herb relieves depression, helps in the treatment of rheumatism, heals wounds and increases appetite. Hymenocrater is a medicinal plant that Iranians have used in the past to improve heart function. This perennial plant grows on mountainous slopes and gives it special beauty thanks to beautiful flowers in spring. The properties of this plant include rheumatism, arthritis, gastrointestinal tract enhancement, liver blocker, body swabs, especially limb edema, epilepsy treatment and nervous system improvement. Figure 1 shows their photos in the Arak area. It is necessary to determine the trace elements, including toxic and necessary elements in food stuff. Thus, increasing or decreasing of them can cause serious diseases in the body (Michenaud-Rague et al. 2012). The amount of elements in each plant varies depending on soil composition, geographical conditions and environmental pollution, and the type of plant (Rahimi and Rabani 2010). In this study, the

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(a) (b)

Fig. 1 Photos of two types of studied herbal plants. **a** Stachys lavandulifolia, **b** Hymenocrater

concentrations of Al, Br, Ca, Cl, Fe, Mn, Mg, Na, Sc and Zn in two species of Hymenocrater and *Stachys lavandulifolia* were measured using instrumental neutron activation analysis (INAA) method.

2 Methodology

2.1 Sampling and sample preparation

In this study, 12 herbal samples from 6 regions of Arak, Shazand and Alvand Mountain in Hamadan in Iran were collected and washed with double-distilled water and dried. The samples were dried in the open air and then placed in an oven at 80 °C for 4 h to prevent weight change during the measurement. Samples were ground using a ceramic mortar to obtain a fine-grained powder similar to a standard sample. A small amount of each sample (30–57 mg) was sealed in polyethylene cans. Samples were coded according to the first letter of the scientific name of the herb and the location of sampling. Therefore, S and H denote two herbs, and A, S, R, L, GH and SH denote the domain of the Alvand, Sefidkhani, Rasvand, Lajvar, Ghoroghdar and Sharra mountains in the provinces of Markazi and Hamadan, respectively. Standard reference V-10 weighing 49.8 mg was also prepared under similar conditions. Using a pneumatic transfer system, each sample was irradiated together with standard reference material in a research reactor in Tehran. The thermal neutron flux was at the sample location 3×10^{13} n cm^{-2} s^{-1} (Khalafi and Rahmani 2008). The gamma ray spectra of samples and standard reference materials were recorded immediately after irradiation to determine radioisotopes with a shorter half-time decay period such as Al and Mg. For the determination of Ca, K, Mn, Cl and Na, gamma ray spectrum was recorded 15 min after irradiation. To determine the elements with a longer half-life, the irradiation was carried out for two hours, and counting was done after a week. In Table 1 is listed the selected radioisotopes used to determine the amount of elements and gamma energy as well as the decay time of radioactive nuclei (IAEA-TECDOC-564 1990). Gamma spectra of the samples were recorded using the high-purity germanium detector (HPGe) Model 5574 EGPC manufactured by the Intertechnique Company of France with 10% relative efficiency along with multichannel analysis. The energy resolution of this detector was 1.95 keV for a gamma line corresponding to 1332.520 keV belonging to ^{60}Co. The detector and preamplifiers were shielded in a three-layer shield consisting of 10 cm lead, 1 mm cadmium and 2 mm copper. This shield is useful for reducing background radiation. Cosmic soft beams, which contain photons and electrons, are reduced to a very low level by the lead shield. X-rays (73.9 keV), produced by the interaction of cosmic radiation with lead, are effectively absorbed by the copper layer; also, the cadmium layer absorbs thermal neutrons produced by the cosmic ray and prevents it from entering the detector crystal (Azizi 1981). An example of the spectra recorded for the sample of Hymenocrater with code HR is shown in Fig. 2. The concentration of elements in the activated samples was quantified using the winspan2004 gamma spectrum analysis software package. This software uses Eq. (1) to determine the concentration of elements in the sample based on a comparison with the value given in the reference material (Thomson 2000).

$$C_s = C_{st}\left(\frac{A_s\left(e^{-\lambda t_d}\right)_{st}}{A_{st}\left(e^{-\lambda t_d}\right)_s}\right), \quad (1)$$

where in this equation C_s and C_{st} are the unknown and known concentration of the element (target) in the sample and standard, and A_s and A_{st} are also the count under known photopeak for the sample and reference material, respectively, and td is the decay time. $\left(e^{-\lambda t_d}\right)_s$ and $\left(e^{-\lambda t_d}\right)_{st}$ are decay factors for the element in the sample and standard sample.

For quality control, the reference material V10 was analyzed, statistical analysis was carried out and the value of the Z-score was calculated at the confidence level of 0.95 according to Eq. 2 (Bode and Dijk 1997). The result of this analysis is listed in Table 2.

Table 1 Physical parameters of radioisotopes using for elemental analysis of samples

Elements	Radioisotope	Half-life	Selected gamma ray energy (keV)	Irradiation condition
Al	^{28}Al	2.24 m	1778.969	Exposure time: 2 min. Recorded gamma spectra immediately after irradiation for 300 s
Mg	^{27} Mg	9.46 m	1014.52	
Ca	^{49}Ca	8.718 m	3180.317	Exposure time: 2 min. Recorded gamma spectra after 15 minutes for 900 s
Cl	^{38}Cl	37.24 m	1642.66	
K	^{42} K	12.40 h	1524.67	
Mn	^{56}Mn	2.58 h	1810.757	
Na	^{24}Na	14.997 h	1368.62	
Br	^{82}Br	35.4 h	654.75	Exposure time: 2 h. Recorded gamma spectra after one week for 80,000 s
Fe	^{59}Fe	44.63 d	1099.245	
Zn	^{65}Zn	243.93 d	1115.549	

Fig. 2 Registered gamma spectrum for herbal plants encodes HR samples for 900 s

Table 2 Comparing the reports and the measured values for the V-10 quality control sample and the calculated Z-score Element symbole

	Average value reported (mg/kg)	Acceptable range with 95% confidence (mg/kg)	Measured value (mg/kg)	Z-Score value
Al	47	30–87	53±11	0.39
Br	8	7–11	6.5±0.8	−1.39
Ca	21,600	21,000–22,200	23625±1556	2.43
Fe	186	177–190	188±5	0.49
Mg	1360	1330–1450	1390±25	0.92
Mn	47	44–51	52±1	2.75
Na	500	440–570	595±75	1.91
Sc	0.014	0.012–0.015	0.011±0.006	−0.970
Zn	24	23–25	23.6±0.9	−0.59

$$z = \frac{(M - R)}{\sqrt{E_M^2 + E_R^2}}, \quad (2)$$

where M is the measured value, R is the reported value mentioned in the reference spreadsheet, and E_M and E_R are the uncertainty of the measured and reported values, respectively. The allowed value of Z should be in the range from −3 to 3.

The accuracy and precision of the measurements were assessed by comparing the results of measuring the concentrations of the elements, and the values given in the reference material using the IAEA criteria in order to estimate the uncertainty of measuring the concentration of elements in the samples and the components of uncertainty including weighting uncertainty and counting uncertainty under the full energy peak were taken into account. Accordingly, measurement accuracy is acceptable if (Bode and Dijk 1997):

$$\left|\text{Value}_{\text{IAEA}} - \text{Value}_{\text{analyst}}\right| \leq 2.58 \times \left(\text{unc}_{\text{IAEA}}^2 + \text{unc}_{\text{analyst}}^2\right)^{1/2} \quad (3)$$

The accuracy of measurements is acceptable if the equation of 3 is satisfied (Thomson 2000).

$$\sqrt{\left(\frac{\text{unc}_{\text{IAEA}}}{\text{value}_{\text{IAEA}}}\right)^2 + \left(\frac{\text{unc}_{\text{analyst}}}{\text{value}_{\text{analyst}}}\right)^2} \times 100 \leq 20, \quad (4)$$

where the subscript and IAEA analyst are the value reported by the IAEA and measured in this experiment, respectively. For example, this value for Ca, Fe, Mg and Na was obtained as 7.2, 2.6, 1.8 and 15, respectively. In this study, reference materials V-10 and SL-1 were used as standard and quality control samples, respectively.

3 Results and Discussions

Table 3 presents the results of the measurement of the concentration of ten elements for 12 specimens of two species of medicinal herbs called Hymenocrater and *Stachys lavandulifolia* (6 of each). In Table 3, the sample code for (*Stachys lavandulifolia*) and (Hymenocrater) began with the letters S and H, respectively.

Table 3 shows the amounts of useful and necessary elements in the human body, including magnesium, calcium, manganese, iron and zinc in Hymenocrater samples and *Stachys lavandulifolia* taken from different areas of Arak and Hamadan zones. In this study, it was observed that the highest concentration belongs to magnesium (5560), calcium (1860), iron (4860), zinc (5560) and manganese (2780) in mg/kg. The study shows that the herbs analyzed contain significant amounts of useful elements, such as zinc, iron, calcium, potassium, manganese, sodium and chlorine, which play an important role in maintaining the body's health. Iron plays a key role in providing oxygen to cells, metabolism and normal body growth (Institute of Medicine 2001), while calcium and magnesium are essential for bone strength, muscle contraction and neurotransmission (WHO 2019; HHS 2019); also, zinc has an important role in cell division, muscle contraction and neurotransmission (HHS 2019). Manganese helps in the production of collagen in the skin, wound healing, metabolizing fats and carbohydrates (Institute of Medicine 2001). Manganese is one of the most abundant metals in the Earth's crust, usually occurring with iron. Manganese is an essential element for humans and other animals. A deficiency or exceeding a certain amount can cause side effects. The permissible manganese limit for an adult is 0.06 mg/kg body weight, which is calculated based on a daily intake of 11 mg of this element. With daily consumption of less than this amount, no side effects were observed (WHO 2019). Assuming that all the manganese from these plants is absorbed by the body, and considering that the people of this region consume a maximum of about 10 g of these plants per day, their intake does not exceed ten milligrams. For the SGH sample only, it may be more than 11 mg (27.8 mg), but not more than 1.46 mg for other samples. This means that, in addition to helping you provide the body you need, it does not exceed the permissible amount. For zinc, the estimated average requirement (EAR) is 4.9 mg for men and 6.8 mg for women, which is no more than 0.55 mg of the HSH sample, while the recommended daily allowance (RDA) is 11 mg for men and 8 mg for women (Institute of Medicine 2001). Iron functions as an ingredient in a number of proteins, including enzymes and hemoglobin, which are important for transporting oxygen to tissues throughout the body for metabolism. The daily intake of iron is estimated about 16 to 18 mg for men and 12 mg for women, with an upper limit (UL) of 45 mg (Institute of Medicine 2001). Overdosage can cause gastrointestinal disorders. If swallowed, 10 g of SH sample approximately 11 mg of the daily requirement may be provided. Calcium is the richest mineral in the body and it is estimated that a healthy body needs 1000 mg per day to meet its nutritional needs (98%) (Heaney 2002). In the case of SR sample ingestion about 10 g, this intake estimated a maximum of about 180 mg/day. The amount of aluminum, bromine and scandium recommended intake, and their effect on organism function are not known, but the tolerable intake for each of them is approximately 70 mg daily (WHO 1996). For 10 g SSH and HR samples, the maximum intake of Al and Br is 39.6 mg and 0.18 mg, respectively. The Sc value in the samples was very low, so it does not pose a health risk. The samples of HA, SA, SGH and HGH were collected from the slopes of an igneous mountain, where their soil is richer in chlorine, magnesium, manganese, sodium and scandium

Table 3 Elemental concentration in herbal plants in mg/kg

Sample code	Concentration of measured elements in mg/kg									
	Al	Br	Ca	Cl	Fe	Mg	Mn	Na	Sc	Zn
SS	1210 ± 60	0.99 ± 0.07	13,700 ± 152	405 ± 20	387 ± 10	2180 ± 46	78.7 ± 2.7	313 ± 25	0.05 ± 0.01	44.8 ± 1.7
SR	797 ± 28	10.10 ± 0.52	18,600 ± 669	296.0 ± 8.6	336 ± 6	2750 ± 50	57.2 ± 1.4	306 ± 9	0.26 ± 0.03	45.2 ± 1.6
SL	603.2 ± 6.6	14.10 ± 0.80	11,400 ± 319	338 ± 25	475 ± 10	2140 ± 75	50.2 ± 0.9	468 ± 22	0.27 ± 0.03	31.9 ± 1.2
SGH	109 ± 11	12.50 ± 0.50	11,100 ± 455	344 ± 19	146 ± 6	1940 ± 178	2780 ± 125	692 ± 69	0.06 ± 0.01	37.1 ± 1.2
SSH	3960 ± 281	0.90 ± 0.09	13,200 ± 1029	337 ± 11	332 ± 10	5540 ± 100	79.6 ± 1.5	283 ± 8	0.044 ± 0.002	52.2 ± 1.6
SA	493 ± 74	12.90 ± 0.76	11,100 ± 1132	334 ± 21	468 ± 13	2260 ± 117	43.8 ± 1.7	399 ± 10	0.02 ± 0.00	32.9 ± 1.5
HA	1250 ± 78	11.20 ± 0.35	15,600 ± 858	115.0 ± 4.7	415 ± 7	4390 ± 140	108 ± 5	331 ± 22	0.06 ± 0.01	48.2 ± 1.4
HL	580 ± 20	13.20 ± 0.63	17,500 ± 577	288 ± 16	454 ± 9	3620 ± 185	62.5 ± 0.9	549 ± 28	0.27 ± 0.02	50.4 ± 1.4
HR	777 ± 25	18.30 ± 0.80	12,000 ± 588	215 ± 9	486 ± 13	3410 ± 65	55.3 ± 1.1	259 ± 24	0.51 ± 0.03	28.4 ± 1.6
HS	3400 ± 245	0.71 ± 0.06	16,900 ± 828	251 ± 9	1090 ± 35	2250 ± 71	146 ± 6	452 ± 17	0.88 ± 0.06	31.3 ± 1.3
HSH	685 ± 38	12.60 ± 0.78	11,200 ± 985	486 ± 32	258 ± 8	4230 ± 258	55.2 ± 1.4	286 ± 10	0.025 ± 0.00	55.6 ± 1.5
HGH	621 ± 35	10.50 ± 0.50	15,100 ± 1389	498 ± 27	169 ± 7	4650 ± 158	59.8 ± 2.1	735 ± 19	0.09 ± 0.01	45.2 ± 1.5

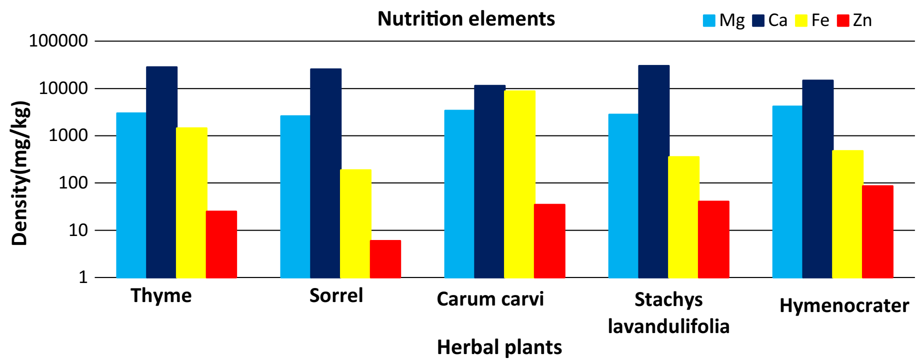

Fig. 3 Comparison of Ca, Fe, Mg and Zn concentration in some herbal plants

than other soil samples taken from limestone slopes (Ayoubi and Adman 2019; Yousefi Fard et al. 2012). However, in terms of the richness of important elements such as iron, magnesium and zinc, no significant differences were observed with the other samples. A comparison of some important elements between green tea, thyme, sorrel, Hymenocrater and Stachys lavandulifolia is shown in Fig. 3 (Suliburska et al. 2012; Kashian et al. 2018), indicating that magnesium was more in Hymenocrater and Stachys lavandulifolia than other herbal plant. Therefore, these plants can compensate for the lack of Mg in the body, which improves the function of the nervous system.

4 Conclusion

In this study, 12 samples of two species of medicinal plants (Hymenocrater and *Stachys lavandulifolia*) were analyzed using a neutron activation method. The experiment has shown that nutrients such as magnesium, manganese, zinc, calcium and iron are present in good amounts in these plants that can provide the body with the necessary elements. Plants growing near the plutonic mountains are richer in Mg and Zn compared to Limestone Mountains.

Acknowledgements The research was funded by the Research Council of Arak University and the Atomic Energy Organization of Iran, so the authors express a lot of gratitude.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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