

DETERMINATION OF SOME MACROELEMENTS IN REPRESENTATIVES OF GENUS THYMUS L. BY ATOMIC ABSORPTION SPECTROMETRY

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SUMMARY: Two procedures for mineralization in some representatives of genus *Thymus* L. and determination of Ca, Mg, K, Na, Fe, Zn and Mn by atomic absorption spectrometry are suggested. The proposed procedures are checked by the method of standard additions. The obtained results indicate a satisfactory value for the recovery ranging from 98.0 to 101.9%. In thirty samples of genus *Thymus* L. collected in various parts of Macedonia, the content of the above mentioned elements was determined. A large diversity in the composition of macroelements is probably due to the diverse origin, climate, ecological and other factors.

Key Words: Thymus, Macroelements, Ca, Mg, K, Na, Fe, Zn, Mn, Determination, Atomic absorption spectrometry

Some representatives of genus *Thymus* L. (*Thymus vulgaris* and *Thymus zygis*) present a certain biological resource of drug *Thymi folium*, being used in the form of secondary medicinal herbs such as expectorants [1]. Very often some other representatives of this genus are manifested as substitute of *Thymi folium*. However, in the flora of the Republic of Macedonia *Thymus tosevii*, *Thymus longidens*, *Thymus moesiacus* and other samples of genus *Thymus* appear in great deal [2], known as - thyme, although it refers to many other quite different species appearing with their own subspecies, variations and forms.

Thyme is very old medical herb and is a very favorite medicine in the folk medicine. It has been used such as stomach, spasmolytic, antiseptic, expectorants, and antihelminthic, i. e. as substitute for *Thymi folium* drug. The dry upper part of the herb is being used in form of tea drink (water extract) [3].

The data determining the mineral content (macro and micro elements) are very poor in the representatives of the genus *Thymus* [4 - 6]. Considerate the meaning of some macroelements, the aim of this work is to include a suitable procedure for determining of some macroelements (Ca, Mg, K, Na, Fe, Mn, and Zn) in the genus *Thymus* and to determination these elements in representatives of genus *Thymus* L. in the flora of Republic of Macedonia by atomic absorption spectrometry.

Material and Methods

Instrumental

A Perkin-Elmer Model 370 atomic absorption spectrometer was used. The optimal conditions for

Ca, Mg, K, Na, Fe, Zn, and Mn determination are given in Table I. An acetylene-air mixture was used as combustion gas.

Table I
Instrumental parameters for determination of Ca, Mg, K, Na, Fe, Zn, and Mn in representatives of genus *Thymus* by atomic absorption spectrometry

Element	Wavelength (nm)	Slit (nm)	Lamp current (mA)	Linear working range (mg. mL ⁻¹)
Ca	422.7	0.7	15	0 - 4.0
Mg	285	0.7	15	0 - 0.4
K	766.5	2	12	0 - 4.0
Na	589	0.7	8	0 - 0.5
Fe	248.3	2	30	0 - 5.0
Zn	213.3	0.7	15	0 - 0.5
Mn	279.5	0.2	20	0 - 3.0

Reagent and samples

All reagent and standard substances were of analytical grade. Standard solutions with mass concentration 1 gL⁻¹ were prepared from commercial solution of CaCl₂, MgCl₂, KCl, MnCl, FeCl₃, ZnCl₂, and MnCl₂ (Merck, Darmstadt). The standard solutions with lower concentrations were prepared by diluting with redistilled water. Samples of different species of the *Thymus* genus are being gathered from different areas of Macedonia. The same has been milled before using in some examinations.

Mineralization procedures

1. *Dry procedure.* - 1 g of milled drug was transferred into a porcelain crucible and heated on a hot plate for one hour, then in muffle furnace at 150 °C for 30 minutes, and finally at 500 °C for 6 hours. After cooling at room temperature, the obtained mineral residue was dissolved in 100 ml of 4 % solution of HNO₃.

2. *Wet procedure.* - 1 g of milled drug transferred into a small Erlenmeyer flask, wetted with 15 ml concentrated HNO₃ and heated in a boiling water-bath for two hours, covered with glass-watch. Then, the heating was continued on hot plate until it got almost dry. The obtained mineral residue was dissolved in 100 ml 4 % solution of HNO₃.

Results and Discussion

In the methods for macroelement determination in the plant material, one of the more important stage is mineralization of samples and transforming the elements in solution. The priority of dry procedure against wet procedure analysis is in the fact that, it is more simple and contamination of the sample is avoided, because reagents are not used in the process of ashing, it is possible to take greater amount of the sample, but on the other side the procedure lasts longer and failures of the easy evaporated elements is possible [8, 9]. In our examinations two ways of mineralizations are being applied. However, with the dry procedure ashing at 500 °C is done were it had been stated that there were not losses (Table II). To avoid contamination of the treated solution, using wet procedure, mineralization with HNO₃ is applied, getting completely dry and complete dissolving with HNO₃ solution (4 % w/w). The results of macroelements determination in the representatives of genus *Thymus* with dry and wet mineralization are given in Table II.

Table II

Content of Ca, Mg, K, Na, Fe, Zn and Mn in representatives of *Thymus* genus by atomic absorption spectrometry, presented according to the two mineralization procedures

Element	Procedure 1		Procedure 2	
	Content/mg.g ⁻¹	*RSD (%)	Content/mg.g ⁻¹	*RSD (%)
Ca	6.34	3.70	6.86	3.72
Mg	2.08	4.18	2.09	3.5
K	6.23	3.75	7.84	2.33
Na	0.08	3.6	0.09	2.9
Fe	0.31	3.08	0.43	2.06
Zn	0.04	2.74	0.03	2.97
Mn	0.11	3.43	0.13	3.84

Accuracy of the procedures, presented by the relative standard deviation, was satisfactory for the examined elements and for the both procedures. The appliance of the dry mineralization procedure, the accuracy is smaller in ore elements.

The above mentioned procedures were checked by the method of the standard additions, as well. Recovery value (R %) for Ca range from 98.9 - 102.5 %, for Mg from 98.9 - 103.3 %, for K from 98.0 - 102.0 %, for Na from 98.2 - 101.5 %, for Fe from 98.7 - 100.5 %, for Zn from 98.5 - 101.8 % and for Mn from 97.8 - 102.7 %. As it can be seen, the recovery values vary from procedure to procedure as well as from element to element. This variations could be the result of various interferences from the matrix onto the investigated elements [10]. According to the obtained values for R (%) it can be concluded that the both procedures can be useful for determination of macroelements in the representatives of genus *Thymus*.

With appliance of the wet procedure to mineralization of the given samples, Ca, Mg, K, Na, Fe, Zn and Mn is determined in 30 *Thymus* samples, collected in various parts of Macedonia. The results of this examinations are given in Table III.

In all examined samples of genus *Thymus* L., Ca (4.7 - 10.20 mg.g⁻¹), Mg (1.85 - 3.60 mg.g⁻¹) and K (3.30 - 19.12 mg.g⁻¹) are presented in largest amounts. The content of Na was between 0.07 and 0.26 mg.g⁻¹. The content of Fe from 0.10 and 0.97 mg.g⁻¹ (except of one sample which contained 3.81 mg.g⁻¹), Zn from 0.026 - 0.084 mg.g⁻¹ (except of one sample which contained 0.119 mg.g⁻¹) and Mn from 0.026 - 0.287 mg.g⁻¹.

Calcium, Mg, K, and Na are physiological plant macroelements and are always present in great quantities in the plants [11]. According to the ground on which they grow, the content and relation of (Ca + Mg) to (K + Na) can be various [12]. Their content in this examined material is expected. On the other side, Fe, Zn, and Mn are content variously in various plants. So that, there are different kinds having tendency to accumulate Fe (13), Mn (14) and other elements, and such ones containing minimal quantities of this elements. The representatives of genus *Thymus* L. included in this research have a tendency to accumulate Fe which in some taxons (*Thymus albanus*) is present in enormous big quantity (3.81 mg.g⁻¹). Determination of this elements (Fe, Zn and Mn) in representatives of genus *Thymus* is justified and they present decisive factor in increasing the content of the active constituents of this plants (by up to 20 %) [5].

In representatives belonging to the same species, collected in various places there is variations concerning the content of some elements. So, *Thymus tosevii* shows great variations in Ca and Fe content. *Thymus tosevii* and *Thymus albanus* in K, Fe

Table III
The content of Ca, Mg, K, Na, Fe, Zn, and Mn in representatives of *Thymus* genus determined by atomic absorption spectrometry (results are given in mg.g⁻¹)

№	Samples	Elements						
		Ca	Mg	K	Na	Fe	Zn	Mn
1 2 3 4 5 6	<i>Thymus tosevii</i> Velen. ssp. <i>tosevii</i> var. <i>tosevii</i>							
	- v. Nicolič, 1994	6.57	1.84	15.29	0.14	0.34	0.037	0.063
	- v. Laki, 1994	5.71	2.53	14.80	0.10	0.12	0.029	0.090
	- v. Vitačevo, 1995	7.91	2.28	12.35	0.09	0.21	0.026	0.072
	- v. Tajmište, 1995	6.89	2.78	11.04	0.14	0.58	0.037	0.092
	- v. Majdan, 1995	7.65	2.83	12.78	0.13	0.16	0.033	0.026
7	- v. Rajko Žinzifov, 1995	10.20	2.42	11.57	0.09	0.40	0.064	0.056
8	<i>Thymus tosevii</i> Velen. ssp. <i>tosevii</i> var. <i>longifrons</i> Ronn.							
	- Karadžica, 1994	5.43	3.20	19.12	0.10	0.36	0.036	0.252
9 10	<i>Thymus tosevii</i> Velen. ssp. <i>substriatus</i> (Borb) Matevski							
	- v. Nikolič, 1994	5.43	2.43	16.57	0.10	0.20	0.035	0.098
	- v. Vitačevo, 1994	6.57	1.84	15.29	0.14	0.34	0.037	0.063
10	- v. Vitačevo, 1995	7.65	2.41	12.78	0.14	0.29	0.084	0.066
11 12 13	<i>Thymus tosevii</i> Velen. ssp. <i>tosevii</i> var. <i>degenii</i> (H. Br.) Ronn.							
	- Preslap, 1994	5.57	2.47	15.98	0.10	0.14	0.044	0.285
	- Lazaropole, 1995	8.16	3.20	5.39	0.09	0.53	0.119	0.167
13	- Vrben, 1995	7.65	2.52	8.17	0.07	0.48	0.034	0.138
14 15 16	<i>Thymus alsharensis</i> Ronn							
	- v. Majdan, 1994	5.71	2.93	14.90	0.11	0.56	0.038	0.061
	- v. Majdan, 1995	7.91	3.51	15.65	0.08	0.10	0.057	0.042
16	- v. Majdan, 1995	8.80	3.26	13.65	0.10	0.18	0.058	0.033
17 18 19	<i>Thymus longidens</i> Velen. var. <i>lanicaulis</i> Ronn.							
	- v. Sonje, 1994	6.71	2.35	19.12	0.12	0.45	0.033	0.047
	- v. Banjani, 1994	7.29	2.43	14.90	0.12	0.79	0.033	0.082
19	- v. Banjani, 1995	10.20	2.66	14.96	0.13	0.80	0.053	0.081
20 21	<i>Thymus longidens</i> Velen. var. <i>dessarecticus</i> Ronn.							
	- Karadžica, 1994	4.57	2.29	12.26	0.11	0.63	0.033	0.237
21	- Karadžica, 1995	6.63	2.44	12.87	0.14	0.97	0.029	0.281
22	<i>Thymus ciliatopubescens</i> - v. Sonje, 1994	7.71	2.30	11.08	0.13	0.53	0.032	0.037
23 24 25	<i>Thymus macedonicus</i> (Degen et Urumov) Ronn.							
	- v. Laki (in bloom), 1994	5.43	2.43	16.57	0.10	0.20	0.035	0.093
	- v. Laki (before bloom), 1994	5.14	2.87	15.88	0.12	0.18	0.082	0.078
25	- v. Laki (in bloom), 1995	7.91	2.64	9.74	0.07	0.28	0.035	0.065
26 27 28 29	<i>Thymus moesiacus</i> Velen.							
	- Popova Šapka, 1994	4.71	3.41	18.24	0.26	0.72	0.032	0.203
	- Bistra, 1994	5.57	2.89	10.29	0.16	0.50	0.035	0.161
	- Popova Šapka, 1995	7.91	3.60	12.17	0.19	0.38	0.048	0.287
29	- Bistra, 1995	7.78	3.07	8.26	0.08	0.90	0.028	0.075
30	<i>Thymus albanus</i> H. Braun - Popova Šapka, 1995	9.44	4.36	3.30	0.10	3.81	0.047	0.274

Zn, and Mn, while *Thymus longidens* var. *lanicaulis* in Ca and Mn content. On the other side plants belonging to different species and collected in the same places (*Thymus tosevii* - *Thymus macedonicus* from v. Laki; *Thymus tosevii* - *Thymus longidens* from Karadžica; *Thymus tosevii* var. *tosevii* - *Thymus tosevii* ssp. *substriatus* from Vitačevo; *Thymus longidens* - *Thymus ciliatopubescent* from v. Sonje) have great content equalization with nearly all elements, except K. Compression of the results at the same places (v. Laki and Bistra), but collected in different periods (1994 and 1995) have great content difference of K (16.57 and 9.74) and Mn (0.161 and 0.075). In general, all examined elements and all representatives of genus *Thymus* it can be noticed that Zn and Na are specially equalized, Ca, Mg, and K are equalized while the greatest content variations were present in Fe and Mn.

Comparing our results obtained for the content of K and Mg in certain *Thymus* species with data

about the content of the same element determined in the representatives of genus *Thymus* in various places in Hungary a certain similarities were noticed. So, K content value in *Thymus tosevii* var. *degenii* (Vrben '95) - 8.17 mg.g⁻¹, *Thymus macedonicus* (v. Laki '95) - 9.74 mg.g⁻¹ and *Thymus moesiacus* (Bistra '95) - 8.26 mg.g⁻¹ were nearly the same with value of 0.99 % (9.90 mg.g⁻¹) obtained of the representatives of genus *Thymus* of Hungary [6]. Mg in the species of *Thymus tosevii* var. *tosevii* and *Thymus tosevii* ssp. *substriatus* was presented with 1.84 mg.g⁻¹ while in the representatives of genus *Thymus* of Hungary with 0.06 % i.e. 0.60 mg.g⁻¹[6].

Difference obtained on the content of some macroelements in the representatives of genus *Thymus* in the flora of Republic of Macedonia are due to various time period of the plant collection, various places as well as to which botanical *Thymus* species they belong. Macroelement content probably depends of climatic, ecological, pedological and other factors.

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