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Thermal decomposition and elemental composition of medicinal plant materials–leaves and flowers Principal component analysis of the results

Marek Wesołowski*, Paweł Konieczyński

Department of Analytical Chemistry, Medical University of Gdańsk, Al. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

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Abstract

Studies on the thermal decomposition and on the elemental composition of commercial raw plant materials used in medicine were performed. The 44 independent samples of leaves and flowers originating from different medicinal plant species collected by Medicinal Plants Works "Herbapol" at various factories in Poland were analyzed. The thermal decomposition was performed using the derivatograph. The content of non-metallic (N, P, S, Cl, I and B) and metallic (Ca, Mg, Fe, Mn, Cu and Zn) elements was determined by spectrophotometric techniques after previous mineralization of samples. In order to obtain more clear classification of the analyzed plant materials, principal component analysis was applied. Interpretation of PCA results for three databases (thermoanalytical, non-metals and metals data sets) allows to state, that samples of leaves and flowers from the same plant species in majority of cases are characterized by similar elemental composition and similar course of their thermal decomposition. In this way, the differences in general chemical composition of medicinal plants raw materials can be determined.

Keywords: Differential thermal analysis; Flowers; Leaves; Medicinal raw plant materials; Metals; Non-metals; Principal component analysis; Thermogravimetry

1. Introduction

In the last years, the thermal methods of analysis have found an application in the investigation of material of biological origin [1]. Cebulak and Pliński [2] performed a preliminary study, which indicates that the DTA can be used for taxonomical investigations in order to establish the systematic membership of certain species of algae based on the differences in their chemical composition. The comparative thermal (DTA, TG, DTG) and chemical studies has also been done by Kaloustian et al. [3] on five mediterranean plants. The investigations confirmed the correlation between the maximum decomposition rate of a plant sample at about 300 °C and its content of cellulose. The continuation of the studies on decomposition of bio-polymers from some mediterranean plants during heating showed that the organic matter in the plant is often responsible for the start of the forest fire [4,5]. Moreover, the decomposition of a plant during the heat is mainly dependent on the cellulose level and slightly on the lignin level.

The interesting studies were performed by Price et al. [6–8], which illustrate the use of micro-thermal analysis to carry out localized chemical analysis of biological specimens in order to determine the

* Corresponding author. Tel.: +48-58-349-31-20; fax: +48-58-349-31-24.
E-mail address: marwes@farmacja.amg.gda.pl (M. Wesołowski).

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distribution of natural products (parthenolide and camphor) in plant leaves. The authors showed that camphor is located within oil cells on the leaf surface. Moreover, the possibility to use the thermal analysis (DSC, TG) for a quick characterization of the chemical changes in the organic matter of composted plant materials was also tested by Dell' Abate et al. [9,10]. Analyzing the whole sample without pretreatment enables that these methods can be used as routine tools in monitoring of composting process.

The aim of this investigation was to find whether any relations exist between the elemental composition of medicinal raw plant materials (leaves and flowers) and the thermal decomposition of these samples, originating from the same plant species. These results may give an answer on whether or not thermal analysis technique can be used as a supporting method in chemical analysis of medicinal plant raw materials, as well as whether or not classification of plant samples according to the taxonomic group, from which they originated, is possible [11,12].

2. Experimental

2.1. Materials

In this study, 44 plant samples were used, 27 samples were leaves from 15 species, and 17 samples were flowers from 10 species. The samples were collected by the Medicinal Plants Works "Herbapol" at various factories in Poland.

The plant material was as follows (plant species and sample numbers are given in the brackets): leaves-Folium Betulae (*Betula pubescens* Ehrh., 1), Folium Farfarae (*Tussilago farfara* L., 2–5), Folium Fragariae (*Fragaria vesca* L., 6), Folium Lauri (*Laurus nobilis* L., 7), Folium Melissae (*Melissa officinalis* L., 8), Folium Menthae piperitae (*Mentha piperita* L., 9–11), Folium Menyanthidis (*Menyanthes trifoliata* L., 12 and 13), Folium Plantaginis lanceolatae (*Plantago lanceolata* L., 14), Folium Ribis nigri (*Ribes nigrum* L., 15–17), Folium Rosmarini (*Rosmarinus officinalis* L., 18–20), Folium Rubi fruticosi (*Rubus caesius* L., 21 and 22), Folium Rubi idaei (*Rubus idaeus* L., 23), Folium Salviae (*Salvia officinalis* L., 24 and 25), Folium Sennae (*Senna officinalis* Roxb., 26) and Folium Uvae-ursi (*Arctostaphylos uva-ursi* L., 27); flowers-Flos Anthyllidis (*Anthyllis vulneraria* L., 1), Flos Calendulae (*Calendula officinalis* L., 2), Flos Ericae (*Calluna vulgaris* L., 3 and 4), Flos Farfarae (*Tussilago farfara* L., 5), Flos Hippocastani (*Aesculus hippocastanum* L., 6–8), Flos Lamii albi (*Lamium album* L., 9 and 10), Flos Lavandulae (*Lavandula officinalis* Chaix, 11 and 12), Flos Millefolii (*Achillea millefolium* L., 13 and 14), Flos Sambuci nigrae (*Sambucus nigra* L., 15 and 16) and Flos Ulmariae (*Filipendula ulmaria* L., 17).

Plant material was dried for the constant weight, after that it was ground and passed through a 1 mm sieve. Until analysis, the samples were stored in closed glass jars.

2.2. Thermal analysis

The DTA, TG and DTG curves of raw plant materials were recorded using the OD-103 Derivatograph (MOM, Budapest, Hungary). The 100 mg plant samples were heated in a platinum crucible under the furnace atmosphere at a heating rate of $5\,^{\circ}\mathrm{C\,min^{-1}}$ up to the final temperature of 900 °C. The α-Al_2O_3 was used as reference material.

Analysis of the DTA curves consists of designating the onset (T_i) and peak (T_p) temperatures of an endothermic effect for the first stage of decomposition and for the two successive exothermic effects, for the second and the third stage. In the case of the TG analysis, the mass losses (Δm) in three successive stages of decomposition were determined. However, the temperature range of the DTG peak (ΔT), peak temperature (T_p) and peak height (h) were designated from the DTG curves.

2.3. Chemical analysis

The content of non-metals was determined after previous mineralization of plant samples. The method of nitrogen determination (N as NH_4^+) was based on the reaction between ammonia and Nessler reagent in the alkaline environment [13]. The determination of phosphorus (P as PO_4^{3-}) consisted of the measurement of its concentration by phospho-molybdenum blue complex using iron(II) as a reducer [14]. Sulphur (S as SO_4^{2-}) was determined turbidimetrically [13]. Barium chloride was used as an agent producing turbidity. Chlorine (Cl as Cl^-) was determined basing on

the reaction with mercury(II) thiocyanate, in which the equivalent amount of thiocyanate ions reacts with iron(III) giving red complex [13,15]. The specific reaction of iodine (I as I_2) with starch was used to measure the iodine concentration [16,17]. The content of boron (B as BO_2^-) was determined based on its reaction with Azomethine H [18,19]. Spectrophotometer Spekol-11 (Carl Zeiss, Jena, Germany) was used for all measurements.

The determination of metals was also preceded by mineralization of plant materials. The samples were heated at 220–240 °C for 3 h. Partly dry-ashed samples were evaporated to dryness with a small volume of mixture of concentrated HNO_3 and H_2O_2. Next, the residue was dry-ashed at 450 °C for 5 h and finally, the ash was dissolved in 0.1 mol/l HCl and diluted with redestillated water.

The iron, manganese, copper and zinc concentrations were determined directly from the solution by AAS using Philips PU 9100 instrument, calcium and magnesium were also determined by AAS after appropriate dilution of the solution. To check for the matrix interference, mixed standards containing trace metals were analyzed.

2.4. PCA calculations

Starting point for the PCA calculations was matrix of the data $\boldsymbol{X}$ with dimensions $n{\cdot}p$, where n—is a number of objects (rows) and p—is a number of variables (columns) [20,21]. In this study, six matrices were constructed: three for leaves and three for flowers. In each matrix medicinal plant samples were used as the rows. Columns were the results of thermal, non-metals and metals analyses of plant samples.

The first matrix contained data sets for the three stages of the thermal decomposition of leaves and flowers—T_i and T_p from DTA, Δm from TG as well as ΔT, T_p and h from DTG curves. The second one grouped data sets as the mean values of N, P, S, Cl, I and B content in the plant samples, and the third one consisted of the mean values of Ca, Mg, Fe, Mn, Cu and Zn content in the same plant samples.

Matrix $\boldsymbol{X}$ is at first standardized, than matrix $\boldsymbol{R}$ is calculated according to it. After further calculations, columns in matrices $\boldsymbol{P}$ and $\boldsymbol{W}$ were obtained, which were called principal components (PC). New matrix $\boldsymbol{P}$ reflects main relations among objects and makes possible classification of the samples, whereas matrix $\boldsymbol{W}$ illustrates main relations among variables and enables their selection.

3. Results and discussion

Qualitative and quantitative chemical content of raw medicinal plant materials may differ significantly not only for species of different origin, but also for plants belonging to the same species [22–25]. Reason for this is not entirely recognized, as for now. There is no doubt, that genetic factors may play a crucial role, and the macro-and micro-environmental conditions, in which any plant grows, are important, too.

The thermal decomposition curves of exemplified samples of leaves and flowers are presented in Figs. 1 and 2. There are illustrating changes in the shape of thermoanalytical curves, as effect of differences in chemical composition of the samples. According to obtained results, the general conclusion can be drawn that the thermal decomposition of analyzed leaves and flowers comes through three common stages. In the first stage, a small loss in mass is observed connected with a wide and shallow endothermic effect on the DTA curve. This peak is probably due to the desorption of water from raw plant material together with the evaporation of volatile components and essential oils. Next, the second stage of decomposition is accompanied with strong exothermic effect on the DTA curve and high mass loss as reflected by the TG and DTG curves. There are due to the destruction and combustion of compounds contained in the plant samples. Charred residue after the destruction of low-molecular compounds is burned finally in the third stage of decomposition. Mineral residue is the final decomposition product of all the leaves and flowers.

The results of thermal decomposition of plant materials under study are listed in Tables 1 and 2. The large number of thermoanalytical data describing DTA, TG and DTG curves is the reason for creation of databases, which contain similar values. This makes serious problems for interpretation, especially in attempt to establish essential differences among thermal decomposition of particular plant samples. The PCA could be a helpful tool for such a case. It allows to transform the distribution of 27 samples of leaves and 17 samples of flowers in multidimensional space, into

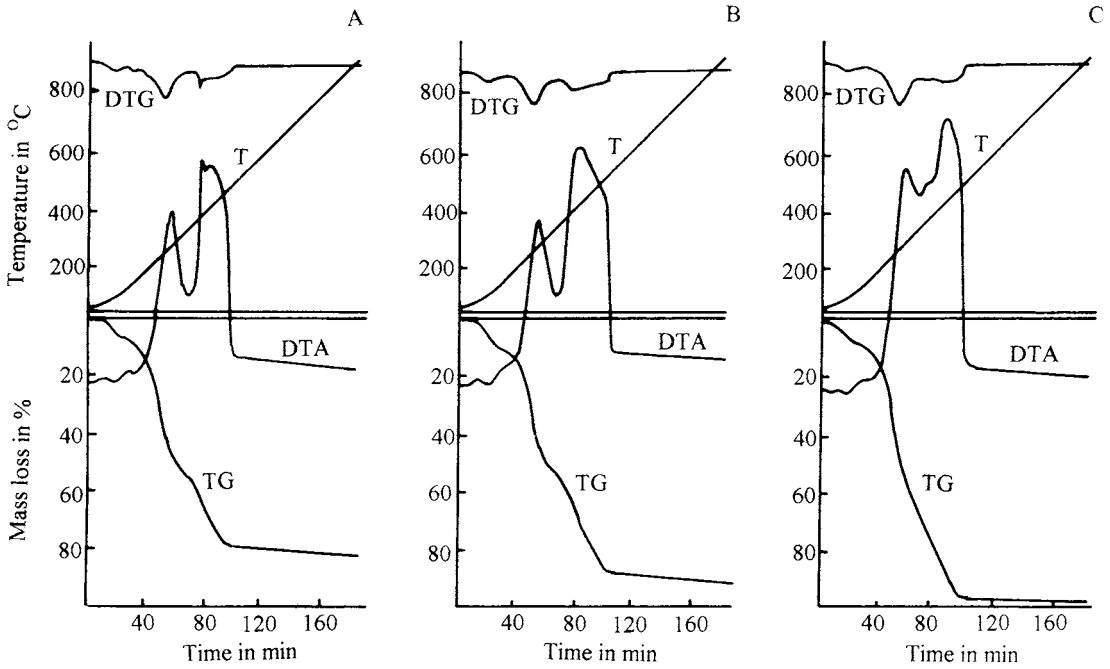

Fig. 1. DTA, TG and DTG curves of the thermal decomposition of leaves: (A) Folium Farfarae (*Tussilago farfara* L., 5); (B) Folium Menthae piperitae (*Mentha piperita* L., 9); (C) Folium Rubi fruticosi (*Rubus caesius* L., 22). Numbers in parentheses denote plant samples compiled in Table 1.

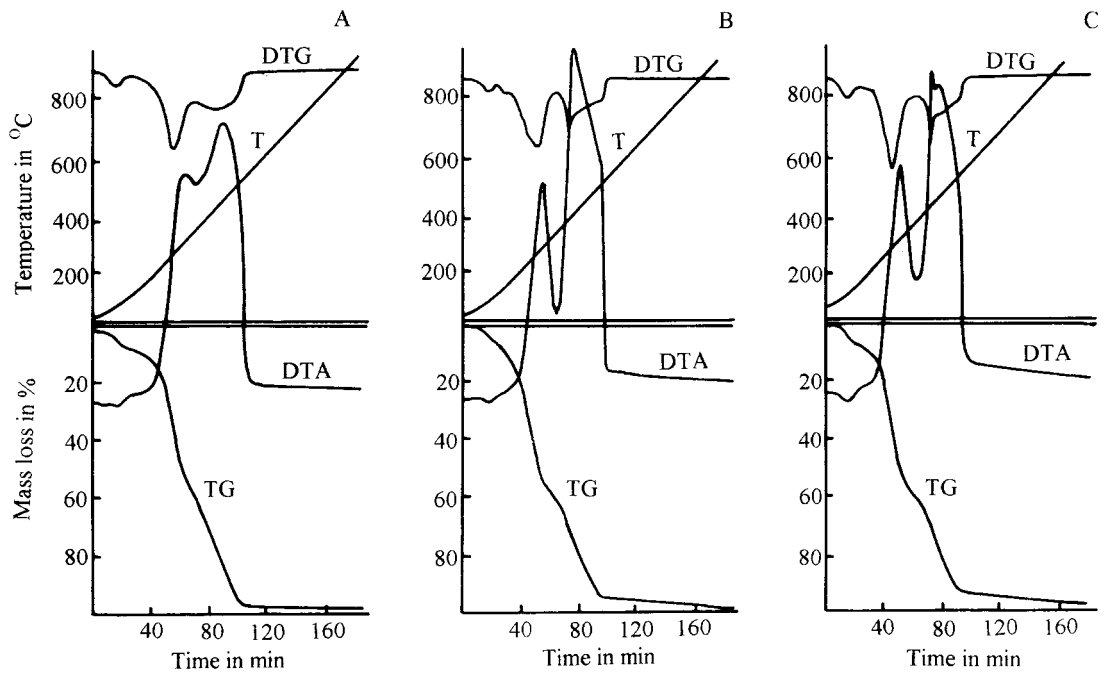

Fig. 2. DTA, TG and DTG curves of the thermal decomposition of flowers: (A) Flos Ericae (*Calluna vulgaris* L., 4); (B) Flos Farfarae (*Tussilago farfara* L., 5); (C) Flos Millefolii (*Achillea millefolium* L., 14). Numbers in parentheses denote plant samples compiled in Table 2.

Table 1
Results of the thermal decomposition of leaves

Sample number	I stage						II stage						III stage					
	DTA (°C)		TG and DTG				DTA (°C)		TG and DTG				DTA (°C)		TG and DTG			
	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)
1	45	85	7.0	95	80	7	160	305	50.5	215	275	25	355	460	40.5	355	455	12
2	30	55	8.5	100	45	6	120	260	42.0	200	230	21	315	385	36.5	330	370	11
3	55	80	7.5	95	75	6	135	285	47.5	230	255	24	350	420	30.0	345	400	13
4	50	90	7.5	105	90	8	150	295	46.5	215	270	27	360	410	31.5	355	410	14
5	30	65	6.0	80	65	5	130	275	48.0	240	245	25	345	420	27.5	330	390	16
6	50	80	7.5	110	70	7	165	290	44.5	170	260	26	305	435	42.5	380	440	13
7	60	85	5.0	80	85	5	165	300	52.0	220	270	25	340	480	39.5	360	415	11
8	55	75	7.5	100	65	7	145	270	48.0	220	240	24	340	415	35.0	360	390	14
9	55	85	9.5	110	80	7	150	275	43.5	200	250	25	340	420	34.5	310	395	13
10	50	75	8.0	95	60	7	155	280	43.5	230	250	23	360	450	37.0	340	420	15
11	65	90	11.0	100	80	9	150	280	43.5	200	260	25	350	420	34.0	330	415	13
12	65	85	6.0	90	65	5	150	280	51.5	210	220	23	345	425	33.0	340	395	13
13	60	90	7.5	95	80	8	165	275	53.5	215	270	31	340	425	31.5	385	435	12
14	55	75	4.5	70	40	5	125	260	49.0	240	215	21	330	405	34.5	330	370	15
15	60	90	7.5	100	80	8	170	295	51.0	220	265	24	380	450	37.5	350	425	21
16	50	70	7.0	80	55	6	145	285	49.0	230	235	21	350	430	36.0	340	395	14
17	60	95	8.0	100	85	8	175	300	52.0	240	265	23	365	445	35.5	330	420	13
18	45	90	8.5	110	75	8	155	305	46.5	210	270	24	360	450	41.5	375	415	13
19	60	90	8.5	110	70	7	145	300	50.0	195	265	24	360	435	41.0	350	400	13
20	50	75	7.0	90	65	6	140	290	50.0	220	255	23	345	440	40.0	370	405	14
21	55	90	8.5	90	80	8	175	295	48.5	195	285	23	355	435	42.0	350	405	15
22	50	75	7.0	80	60	5	150	280	52.5	195	245	29	340	425	36.0	385	400	12
23	55	90	7.5	90	70	8	165	290	53.5	230	250	29	355	430	33.0	380	390	12
24	55	75	7.0	85	60	6	135	270	50.0	215	230	31	335	370	32.0	370	365	21
25	45	85	7.0	90	75	6	160	285	50.0	210	250	27	340	400	33.0	385	380	15
26	50	75	7.0	100	75	6	155	295	53.5	225	255	23	365	440	33.0	395	430	16
27	50	90	7.0	100	85	8	170	305	50.5	190	280	24	330	415	42.0	330	405	13

There are the onset (T_i) and peak (T_p) temperatures from the DTA peaks, the mass loss (Δm) from the TG curves, and the temperature range (ΔT), peak temperature (T_p) and height (h) from the DTG peaks for three consecutive stages of decomposition of plant samples.

Table 2
Results of the thermal decomposition of flowers

Sample number	I stage						II stage						III stage					
	DTA (°C)		TG and DTG				DTA (°C)		TG and DTG				DTA (°C)		TG and DTG			
	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)	T_i	T_p	Δm (%)	ΔT (°C)	T_p (°C)	h (mm)
1	35	65	6.0	75	55	5	135	270	54.5	210	260	33	355	395	35.0	380	390	32
2	50	85	5.0	105	70	7	145	295	56.0	245	290	40	355	445	32.5	190	420	21
3	50	100	7.0	115	50	14	170	320	50.0	195	285	61	370	465	42.0	205	470	26
4	55	85	7.0	120	65	10	145	320	50.5	190	290	58	370	460	41.5	195	465	26
5	60	85	5.0	85	55	8	150	295	52.5	220	270	48	365	405	35.0	220	390	35
6	45	75	5.5	90	45	13	130	295	61.5	240	285	44	355	445	26.5	190	445	25
7	50	95	6.5	80	50	12	150	305	51.0	220	300	48	370	430	38.0	220	405	27
8	50	75	5.0	80	50	8	145	295	52.5	240	295	44	355	450	35.5	195	425	25
9	50	95	5.0	85	90	11	155	280	53.0	215	280	44	340	405	35.5	330	410	54
10	50	80	5.5	85	40	11	120	265	51.5	235	240	51	320	395	32.5	330	385	52
11	55	90	7.5	100	95	9	160	300	52.5	215	260	62	360	425	33.0	255	440	52
12	55	75	7.0	90	70	9	150	290	53.0	230	250	62	360	420	33.0	235	430	54
13	45	90	7.0	95	90	14	165	295	52.0	240	295	69	370	420	36.0	180	430	61
14	55	85	7.0	95	60	8	165	295	52.5	230	285	59	370	425	33.5	190	435	56
15	65	100	6.0	70	95	14	180	305	52.5	220	270	44	265	445	35.5	210	430	24
16	65	90	6.0	70	85	11	160	295	51.0	230	290	49	355	450	34.0	215	425	25
17	55	95	7.5	75	100	13	165	315	49.0	240	285	40	370	470	39.0	300	440	28

There are the onset (T_i) and peak (T_p) temperatures from the DTA peaks, the mass loss (Δm) from the TG curves, and the temperature range (ΔT), peak temperature (T_p) and height (h) from the DTG peaks for three consecutive stages of decomposition of plant samples.

Table 3
Results of PCA of matrices for the leaves and flowers

Matrices	Dimension of matrices ($n \times p$)	Number of principal components					
		PC1		PC2		PC3	
		Percent of variance	Eigenvalue	Percent of variance (cumulative)	Eigenvalue	Percent of variance (cumulative)	Eigenvalue
For leaves							
Thermal analysis	27 × 18	36.11	6.50	16.22 (52.33)	2.92	10.97 (63.30)	1.97
Non-metals	27 × 6	26.91	1.61	21.90 (48.81)	1.31	18.12 (66.93)	1.09
Metals	27 × 6	35.59	2.14	29.92 (65.51)	1.80	14.01 (79.52)	0.84
For flowers							
Thermal analysis	17 × 18	36.98	6.66	14.74 (51.72)	2.65	12.51 (64.23)	2.25
Non-metals	17 × 6	51.22	3.07	18.02 (69.24)	1.08	15.49 (84.73)	0.93
Metals	17 × 6	34.01	2.04	25.19 (59.20)	1.51	17.55 (76.75)	1.05

relatively clear three-dimensional plot. According to such a plot, it is possible to try to classify investigated leaves and flowers by their species.

The results of PCA calculations of thermoanalytical data set for leaves are compiled in Table 3. Considering the eigenvalues, the distribution of plant materials can be illustrated in three-dimensional plot of PC1 versus PC2 and PC3, which account for more than 63% of the variance. As it is shown in Fig. 3, lack of big differences in the chemical composition of leaves could be a reason for a similar localization in space of the samples characterized by the close chemical composition. All of the plants that contain large amounts of tannins, from 5 to 19%, are located in the center of the plot. There are samples of Folium Fragariae (6), Melissae (8), Menthae piperitae (9–11), Menyanthidis (12 and 13), Rubi fruticosi (21 and 22) and Uvae-ursi (27). In similar way, samples containing essential oils are located, such as Folium Betulae (1), Rosmarini (18–20) and Salviae (24 and 25), as well as Folium Melissae (8) and Menthae piperitae (9–11), which also contain tannins. However, all of four samples of Folium Farfarae (2–5), which are rich in mucilage are scattered. Trace amounts of mucilage

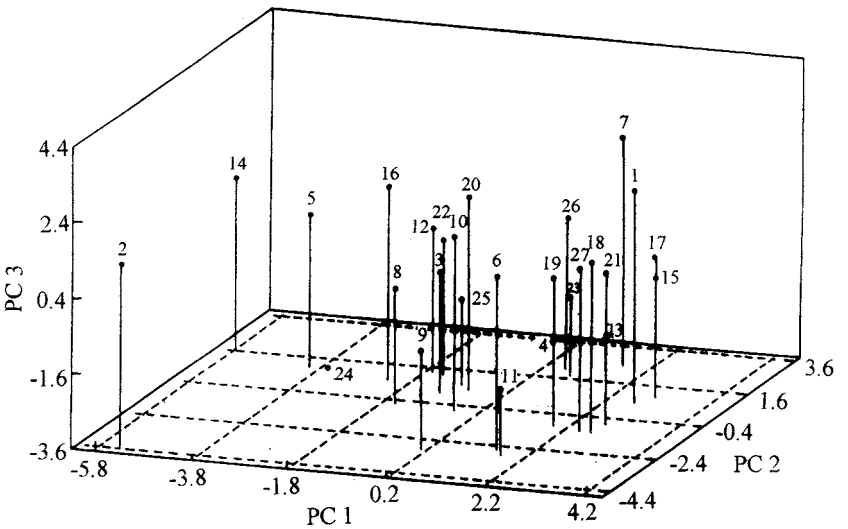

Fig. 3. Plot of the first three principal component score vectors (PC1 vs. PC2 and PC3) for 27 samples of leaves based on the thermoanalytical results compiled in Table 1.

contain also Folium Plantaginis lanceolatae (14) and Sennae (26), of which spatial distribution along PC2 axis is similar to one sample of Folium Farfarae (5). So, it can be stated that the mucilage concentration does not influence the thermal decomposition of plant materials, which could be sufficient for location of samples accordingly to the similar values of PCs.

Analyzing localization of the plant materials from the point of view of the species, from which they were obtained, it is worth admitting that the large number of the species does not make the interpretation easy to do. It is characteristic that three independent samples of Folium Rosmarini (18–20) have similar values of PC1, PC2 and PC3. From three samples of Folium Ribis nigri, two of them (15 and 17) are characterized by almost the same values of PC1, PC2 and PC3, however sample number 16 has different PC1 value. In case of three samples of Folium Menthae piperitae, two of them (9 and 11) are described by similar values of PC2 and PC3, but sample number 10 is characterized by high PC3 value. Similar situation in case of Folium Menyanthidis (12 and 13), Rubi fruticosi (21 and 22), and Salviae (24 and 25) was observed. In each of these plant samples, at least one of the PC's differentiates significantly both of the samples. However, it is possible to state that there are some tendencies indicating relations between course of the thermal decomposition of plant materials and the plant species, from which given material was taken.

The results of PCA calculations of the DTA, TG and DTG data set for flowers are presented in Table 3. Taking into account eigenvalues that were more than 2.0, the distribution of plant samples was illustrated in three-dimensional plot of PC1 versus PC2 and PC3. First three PC's explain together more than 64% of variance of the data. From Fig. 4, one can notice that localization of the samples originating from the same plant species is characterized by narrow range of PC1, PC2 and PC3 values. PC2 appears to be the most differentiating factor for samples from the same plant species. One of the clear examples is Flos Sambuci nigrae (15 and 16). The lowest PC3 values differentiate that samples from the other flowers. Next example of plant raw material, which is characterized by not very high value of PC3 could be Flos Lamii albi (9 and 10). However, it is worth admitting that unless similar values of PC2 and PC3, these two plant samples are differentiated by PC1 value. Two samples of Flos Ericae (3 and 4) are characterized by the highest PC3 value, and their localization according to PC1 and PC2 is outcoming from the other samples.

Analysis of other flower samples revealed that two samples of Flos Lavendulae (11 and 12) and Flos Millefolii (13 and 14) are described by similar values

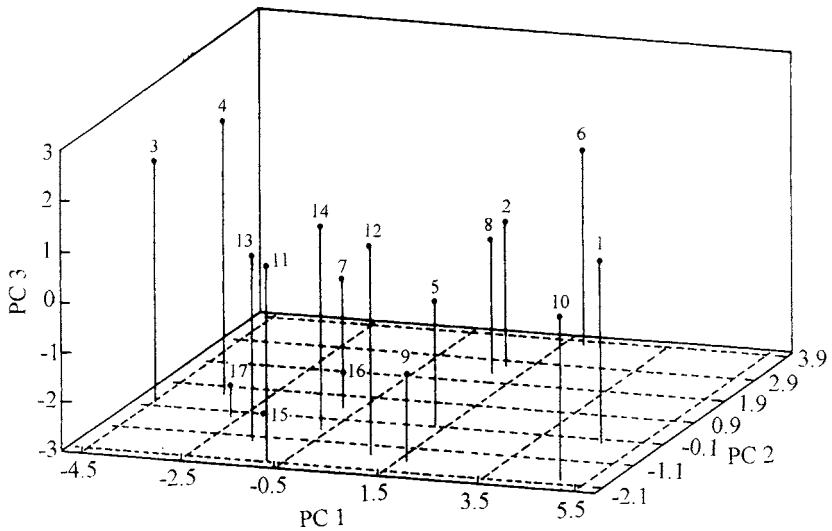

Fig. 4. Plot of the first three principal component score vectors (PC1 vs. PC2 and PC3) for 17 samples of flowers based on the thermoanalytical results compiled in Table 2.

of PC1, PC2 and PC3. In case of Flos Hippocastani (6–8), values of PC2 differentiate all of the three samples in three-dimensional space. It also revealed that the chemical contents of plant samples, which depends on the plant species from which given material originated, influences the course of thermal decomposition of the studied samples.

The results of elements determination revealed that in analyzed material, N had the highest concentration of all non-metals, ranging from several to tens of mg/g of dry plant tissue. The concentrations of P and Cl were on similar levels, about several mg/g of dry material, however, S was found as the element on a relatively small level. The concentration of S varied from tenth parts of mg to several mg/g of dry plant tissue. On the contrary, total I and B levels are significantly lower, their concentrations varied form several to several tens μg/g of dry plant material. On the other hand, the analysis of metals has shown that leaves and flowers contained Ca in the highest concentration, ranging from several to tens of mg/g of dry plant tissue. The concentration of Mg varied from several hundred μg/g to several mg/g of dry plant material. The contents of other metals—Fe, Mn, Cu and Zn in studied plant samples were on the level varied form several to several hundred μg/g of dry plant tissue.

The results of PCA calculations for non-metals and metals data sets are listed in Table 3. General rule after PCA application can be drawn, that according to the distribution of plant materials along PC1, PC2 and PC3 axis, it is possible to separate at least three main classes of samples. They are differentiated because of their contents of elements. Samples of leaves and flowers with low concentration of the analyzed elements are located on the left side of the plot, but samples rich in elements, can be found on the opposite side. In some cases, PCA may also be used to gather together samples of leaves and flowers belonging to the same plant species. Similarly, as in the case of thermoanalytical results, in a narrow range of values of three first PC's can be found all three samples of Folium Menthae piperitae (9–11), Rosmarini (18–20) and Folium Ribis nigri (15–17), along with two samples of Menyanthidis (12 and 13). The same tendency is visible also for flowers. As an example, in close values of three first PC's are located all samples of Flos Hippocastani (6–8), Lavandulae (11 and 12) and Millefolii (13 and 14). It confirms that there are mutual relations between the results of thermal analysis and elemental composition of analyzed material.

4. Conclusions

PCA calculations for three-thermoanalytical, non-metals and metals data sets for leaves and flowers revealed that samples of plant materials originating from the same medicinal plant species in majority of cases are characterized by similar elemental composition and by similar course of their thermal degradation. It reflects close relation between the shape of DTA, TG and DTG curves of leaves and flowers and their chemical composition, which depends among other factors on plant species.

The general conclusion can be drawn that elemental contents together with chemical composition of leaves and flowers samples reflected by the results of their thermal decomposition can be taken into consideration as a factor supporting chemotaxonomy of medicinal plants. However, it should not be expected that PCA interpretation of non-metallic and metallic elements concentrations together with the results of thermal analysis would with no doubt directly classify from which plant species studied material originated from.

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