



# METAL ELEMENTS, ORGANIC AGENTS IN A HERBAL REMEDY, SPECIES *THYMI COMPOSITA*, AND ITS DRUG-CONSTITUENTS

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Beside plant drugs the application of herbal mixtures using in several indication fields is very popular in our time. Since these herbal mixtures contain several bioactive agents, their analysis may cause difficulties. Therefore the description of measurements of components and their standardization is generally missing from the Pharmacopoeias. The examined *Species thymi composita* (STC) is highly effective because of its cholagog, carminative, expectorant, anticatarrhal etc. activity. STC is the mixture of *Althaeae radix et folium* (marshmallow leaf and root), *Verbasci flos* (mullein flores), *Liquiritiae radix* (liquorice root) and *Thymi herba* (thyme). The macroscopic and microscopic identification of components, phytochemical characterization have been described, the element composition of the herbal mixture, plant drugs constituents and teas was studied and the *in vitro* element absorption was examined. It has been stated that the STC could be characterized by its mucilage content and volatile oil composition. STC and its tea contain elements as well and the *in vitro* absorption of some of elements showed relatively high amount. The presence of bioactive compounds, volatile oil components, mucilage and elements may be jointly important in the complex effect, although the relatively higher Mg and Zn content in the tea is particularly relevant that acts via signal transduction process.

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## 1. Introduction

Herbal mixture is one of the best fashionable form of medicinal plant products, firstly in the circle of senior population, considering that medicinal tea contributes to the replacing of liquid in the organism. Beside herbal mixtures putting into circulation by different forms are fundamentally important. The herbal mixtures official in the Formulae Normales VII. (FoNo VII.)<sup>1</sup> and their drug components in the Hungarian Pharmacopoeia VII. (Ph.Hg.VII.)<sup>2</sup>. They are used in numerous indication fields, for example: cholagog, carminative, sedative, laxative, diuretic, antitussive, expectorant and anticatarrhal.

The herbal mixture *Species thymi composita* is official in all (I.-VII.) FoNo prescriptions, although their composition is various and the drug-components –which are directly responsible for the effect- can be found in each composition (Table 1).

The composition of *Species thymi composita* was the richest firstly in FoNo IV. in which 10 dried herbs were applied. Beside the expectorant active *Thymi herba* (thyme

oil), there are *Farfarae folium*, antitussive and antibacterial active *Althaeae folium et radix* (mucilage), *Sambuci flos* (flavonoids), *Verbasci flos* (volatile components, flavonoids and mucilage), antiinflammatory active *Liquiritiae radix*, *Primulae radix* (saponins) and *Papaveris rhoados flos* (alkaloids, anthocyanins), *Pulmonariae herba* (silicic acid)<sup>3-7</sup>. The *Species thymi composita* of FoNo V. contains only 7 drugs and in FoNo VI. 6 drug components are only present. The content of water soluble hepatotoxic pyrrolizidine alkaloids justify the omission of *Farfarae folium*<sup>8</sup>. The *Primulae radix* was also omitted from the tea-mixture regarding the *Primula* species would be declared as protected plants. The composition of *Species thymi* official in FoNo VI. and VII. is the same, they consist of 5 drug constituents containing essential oil (tyme), mucilage (marshmallow and mullein), flavonoids (mullein), saponin (liquorice), as active compounds<sup>9-14</sup>.

The aqueous extract (tea) of *Species thymi composita* prepared by prescription of FoNo has very active catarrh- and cough-resolvent and expectorant activities. Coughing is a complex reflex, which purifies the respiratory tracts from the foreign substances and the secretion formed in excess. In case of cold diseases the coughing irritation is caused by the inflammation of the respiratory mucous membrane so the respiratory secretion is produced in extreme scale which is already not removed by the mucociliaric transport. The secretolytic and secretomotoric activity of expectorant remedies decreased the irritation, consequently they have cough-reliever effect. The thyme belongs to the group of expectorants possessing direct effect mechanism<sup>15</sup>. The essential oil of thyme is excreted in bronchia and increases the bronchus-secretion.

Table 1. Drug constituents (in weight, g) of species *Thymi composita*

Drug constituents of herbal mixture	FoNo IV. (1958)	FoNo V. (1967)	FoNo VI. (1987)	FoNo VII. (2004)
<i>Thymi herba</i>	4	4	14	14
<i>Farfarae folium</i>	4	-	-	-
<i>Pulmonariae herba</i>	4	-	-	-
<i>Althaeae folium</i>	4	4	10	10
<i>Althaeae radix</i>	4	4	10	10
<i>Sambuci flos</i>	4	4	-	-
<i>Liquiritiae radix</i>	4	4	10	10
<i>Primulae radix</i>	4	4	-	-
<i>Verbasci flos</i>	2	2	6	6
<i>Papaveris rhoados flos</i>	2	-	-	-

Since there is no description for studying the mixture *Species thymi composita* (STC), the aim of our work was the pharmacobotanical and phytochemical evaluation of herbal mixture *Species thymi composita*, official in the last FoNo (VII.) and their drug constituents and aqueous extracts (tea), respectively. Therefore, prominently the contents and composition of essential oils obtained from herbal mixture and drugs were studied. Our main research field was the mineral element analysis of herbal mixture, drugs and their aqueous extracts (teas) as well as the study of element absorption in different circumstances.

## 2. Experimental

### 2.1. Plant materials

The following plant drugs were used in the experiment: *Althaeae radix et folium*, root and leaves of marshmallow (*Althaea officinalis* L., *Malvaceae*); *Verbasci flos*, flowers of orange mullein /wooly mullein (*Verbascum phlomoides* L., *Scrophulariaceae*); *Liquiritiae radix*, root of liquorice (*Glycyrrhiza glabra* L., *Fabaceae*); *Thymi herba*, aerial part of common thyme (*Thymus vulgaris* T. *zygis* L., *Lamiaceae*) which was obtained from commercial network (Herbária, Budapest, 2008).

The drug constituents were purchased from trade in cut (scissa) condition. The herbal mixture *Species thymi composita* (STC) was compounded according to the description of FoNo VII.<sup>1</sup>

### 2.2. Extracts

The preparation of aqueous extracts (tea, infusum) of drugs and herbal mixture was the follows: One table spoonful (3-5 g) of herbal mixture is poured off with a cup (300 mL) of hot water and left it to stand for cooling down, then the tea was filtrated (pH=6.2).

The essential oil was obtained by steam distillation, and the determination of essential oil content of herbal mixture and the drugs was carried out by volumetric measuring process according to the Hungarian Pharmacopoeia.<sup>16</sup>

### 2.3. Plant morphology and microscopy

The morphological and microscopical examinations were made on the basis of prescriptions of Hungarian and European Pharmacopoeias (Ph.Hg. VIII., Ph.Eur. 5.)<sup>16,17</sup>.

### 2.4. Mucilage content

For characterization of *mucilage content* of marshmallow root and leaf, and the herbal mixture, as well as the swallow value was determined according to Hungarian Pharmacopoeia (Ph.Hg.VIII.).

### 2.5. Gas chromatographic examinations

The analysis of essential oil composition was made by gas chromatographic (GC) and GC-MS methods: Analysis of composition of essential oils was performed by gas chromatography accomplished with a Fisons 8000 gas chromatograph, equipped with a flame ionisation detector. A Rt- $\beta$ -DEXm capillary column, 30 m long, 0.25 mm id., 0.25  $\mu$ m film thickness was used. Carrier gas was nitrogen at 6.86 mL/min flow rate: 0.2  $\mu$ L was injected (5  $\mu$ L essential oil in 2 mL chloroform). Splitless injection was made at 10 s, split ratio was 1:50. Temperature of injector and detector was 210 °C and 240 °C, respectively. Oven temperature increased with a rate of 8 °C/min from 60 to 230 °C, with a final isotherm at 230 °C for 5 min. Percentage evaluation of compounds was carried out by area normalization; identification of peaks was made by comparison of retention times of standards and co-addition of standards. All measurement was made at duplicate.

GC-MS was performed with a coupled system Agilent 6890N GC, 5973N mass selective detector, the Chrom Card Server Ver. 1.2 equipped with A HP-5MS capillary column, 30 m long, 0.25 mm id., 0.25  $\mu$ m film thickness was used. Carrier gas was helium (pHe was 0.20 MPa), at 1 mL/min flow rate: 1  $\mu$ L (10  $\mu$ L/mL essential oil in ethanol) was injected at 0.7 mg/mL velocity, splitless-type with an Agilent 7683 autosampler. Temperature of injector was 280 °C, temperature of transfer line was 275 °C. Oven temperature was programmed initially at 60 °C for 3 min, then increased with a rate of 8 °C/min to 200 °C, then kept at 200 °C for 2 min and also increased with a rate of 10 °C/min to 250 °C with a final isotherm at 250 °C for 15 min. MS conditions: ionization energy was 70 eV, mass range was 40-500  $mz^{-1}$ , one analysis/min was made. Identification of peaks was carried out by comparison with MS and retention data of standards, and spectra from the NIST library.

### 2.6. Element content

For mineral element analysis, an inductively coupled plasma optical emission spectrometry (ICP-OES) was used by the method of Ladó *et al.* with Spectro Genesis instrument after digestion of the samples with nitric acid (5 mL) and hydrogen peroxide (2 mL)<sup>18</sup>. Se content was determined by square wave voltammetric method using stripping technique with polarographic analyzer (TraceLab 50) by square wave voltammetric measurement on hanging mercury (working) electrode and in the presence of reference electrode (silver/silver chloride) and counter electrode (platinum)<sup>19</sup>.

During the measurement 2 mL sample, 2 mL HCl (1 M) and 2 mL Cu solution (concentration of 5 mg/L) were added to the polarographic cell and the solution was measured.

Table 2. Accuracy of element determination by measurement of Lucerna p-alfalfa reference material standard

	Measured values (mg/kg)	Recovery (%)	Certified values (mg/kg)
Al	325.9±3.6	98.8	330
As	<0.5		0.262
Ba	22.94±0.36	98.0	23.4
Ca	17183±20	98.2	17500
Cd	0.127±0.012	93.4	0.136
Co	0.191±0.017	98.9	0.193
Cr	0.890±0.088	98.8	0.900
Cu	11.27±0.09	96.3	11.70
Fe	321.8±2.1	90.6	355
Hg	<0.3		0.0282
K	17535±16	93.8	18700
Mg	3664±13	104.1	3520
Mn	30.48±0.13	89.1	34.20
Mo	<0.25		0.200
Na	487.6±21.5	102.9	474
Ni	2.87±0.07	112.9	2.54
P	3028±29	99.9	3030
Pb	1.92±0.05	104.3	1.84
Se	0.0501±0.0030	100.2	0.0500
Sr	89.46±0.70	113.6	78.7
V	0.769±0.027	96.1	0.800
Zn	33.51±0.83	100.9	33.2

The accuracy of element determinations was proved by measurements of a certified biological reference material (12-2-03 Lucerna p-alfalfa, Bratislava, Slovakia). The values ranged between 89.1% and 113.6% compared to certified values which show the accuracy of measurements and the well applied digestion method (Table 2). There is no given concentration for Li in the reference material, that's why in case of Li the recovery was determined by the standard addition method where the Li standard was added to one of the samples and measured. The recovery was found to be 95.4%.

### 2.7. In vitro element absorption

The transfer of elements from tea to the stomach (gastric acid of pH=1.1; 1 n HCl (94 g), NaCl (0.35 g) and glycocholl (0.5 g) in 1000 mL of water) and from intestine (pH=6.5; Na<sub>2</sub>HPO<sub>3</sub> (3.9 g) and KH<sub>2</sub>PO<sub>4</sub> (6.1 g) in 1000 mL of water) to plasma (pH=7.5; Na<sub>2</sub>HPO<sub>3</sub> (20.5 g) and KH<sub>2</sub>PO<sub>4</sub> (2.8 g) in 1 L of water)) was studied on a Sartorius membrane diffusion model at 37 °C<sup>21</sup>. The tea (10 mL) and concentrated tea in buffer solution pH=6.5 (10 mL) was poured into a container supplied with a membrane at the bottom of it. The container was plunged into a larger container. The larger outside container contained 100 mL of buffer solution (pH=1.1 or 7.5). Fractions (10 mL) were

taken from the outside container at the following time periods: 30, 60, 90 and 120 min. The aliquot taken was supplied with buffer solution (10 mL, pH=1.1 or pH=7.5 depending on the experiment). The evaluation was omitted in the case of Na at pH=1.1 and of K, Na, P at pH=7.5 because of the K, Na, P content in buffer solutions.

### 2.8. Statistical analysis

Most of the results were expressed in mean ± standard deviation.

## 3. Results

### 3.1. Morphology

The macromorphological characteristics of scissa drug-constituents could be good recognised in herbal mixture. These diagnostical characteristics of each drug are the follows: the white phloem-fibres of *Althaeae radix*, the yellow-brown root pieces of *Liquiritiae radix*, the tomentose leaf-fractions originating from *Althaeae folium*. The yellowish flow-fractions originating from *Verbasci flos*, and final the mixture can be recognised firstly on the bases of penetration order of thyme (Figure 1).

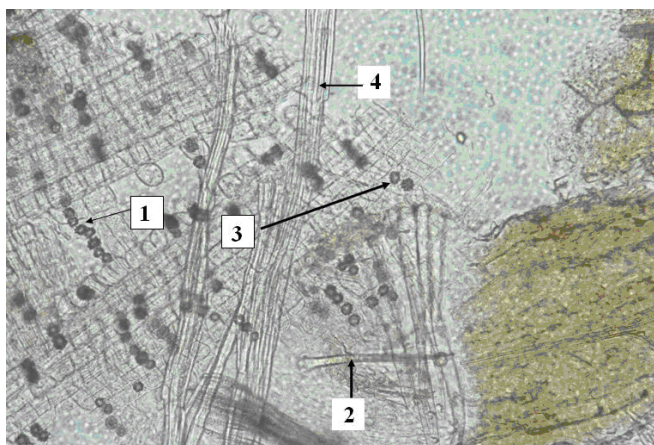


Figure 1. Species thymi composita

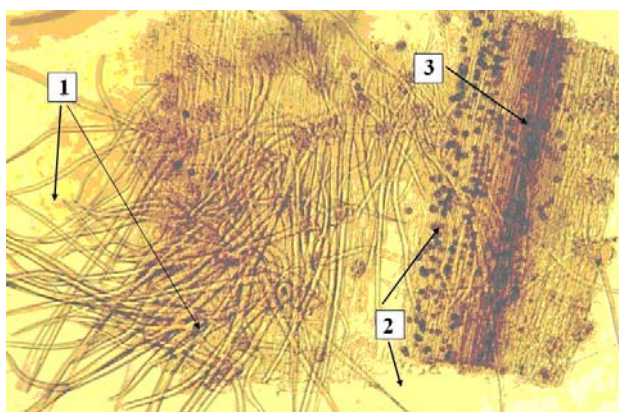
### 3.2. Micromorphology

In micromorphological point of view the main diagnostical characteristics of STC are the star shaped covering hairs of *Althaeae folium* and the branching covering hairs of *Verbasci flos* (Figure 2).

We can find the eye-tooth shaped and hooked covering hairs and *Labiatae trichomes* (glandular hairs) originating from thyme. The calcium oxalate crystals are also characteristics of mixture: rosettes (cluster shaped crystals) from marshmallow and single crystals in fibres of liquorice *radix*. The phloem fibres of *Althaeae* and *Liquiritiae radix* can be also recognizable in mixture powder (Figure 3).

Figure 2. *Species thymi composita* pulvis

1. Liquiritiae radix - fibre containing crystal; 2. *Althaeae folium* - star shaped trichomes, 3. Cluster crystals, 4. *Liquiritiae radix* - fibre bound

Figure 3. *Althaeae folium* pulvis

Diagnostical characteristic: 1. star shaped covering hairs, 2. cluster crystals; 3. vessels

### 3.3. Mucilage content

The swallow value of STC is higher than that of *Althaeae radix* and *folium* (Table 3).

### 3.4. Essential oil content and composition

The contents of essential oil obtained from *Thymi herba*, STC and its infusum are demonstrated in Table 4. The essential oil content of STC is in good agreement with the expectable value, calculated on the basis of essential oil content measured in the thyme leaf. The composition of essential oil obtained from *Thymi herba*, STC and the tea is demonstrated in Table 5 and on the gas chromatograms (Figure 4). In the essential oil of thyme seven characteristic components:  $\beta$ -myrcene, p-cymene,  $\gamma$ -terpinene, linalool, terpinene-4-ol, thymol and carvacrol were identified. These components were detectable in essential oil of the herbal mixture and also in its infusum (tea).

The percentile distribution of the components was similar in oil of thyme and mixture but this rate was changed in the oil of tea: the ratio of polaric phenolic compounds, as tymol and carvacrol increased.

Table 3. Mucilage content

Sample	Swallow value
<i>Species thymi composita</i>	17
<i>Althaeae radix</i>	10
<i>Althaeae folium</i>	7

Table 4. Content of total essential oil

Sample	Oil content (mL/100g)
<i>Species thymi composita</i>	0.36±0.03
<i>Thymi herba</i>	1.3±0.25
Tea of <i>Species thymi composita</i>	0.01±0.001

Table 5. Quantitative characterization of essential oil composition in *Thymi herba*, *Species thymi* mixture and its tea according to gas chromatographic measurements

Components	Percentile distribution (%) of components in essential oil		
	drug	<i>Species thymi composita</i>	
	<i>Thymi herba</i>	herbal mixture	tea
1 $\beta$ -myrcene	1.0	0.7	0.05
2 p-cymene	22.9	19.0	4.6
3 $\gamma$ -terpinene	7.9	6.4	2.8
4 linalool	2.00	2.4	4.3
5 terpinene-4-ol	1.6	1.9	1.9
6 thymol	48.2	52.4	66.1
7 carvacrol	4.1	3.7	5.0

### 3.5. Mineral element composition

The mineral element content of drug components and STC determined by ICP-OES is found in Table 6.

Concentration under the detection limit was obtained for As and Hg in all cases, while for Cd, Co, Li, Pb, Se and V in some cases. At the same time relatively high amount of Li (*Althaeae folium*, *Verbasci flos*, *Thymi herba*), Pb (*Althaeae folium*, *Thymi herba*) and V (*Verbasci flos*) was found compared to the average plant concentrations, which are under 2, 2 and 1 mg/kg, respectively. A slight soil content is shown by the collectively presence of elevated Al (>200 mg/kg), Cr (>1 mg/kg) and Fe (>300 mg/kg) content in *Althaeae folium*, *Verbasci flos*, *Thymi herba* and STC.

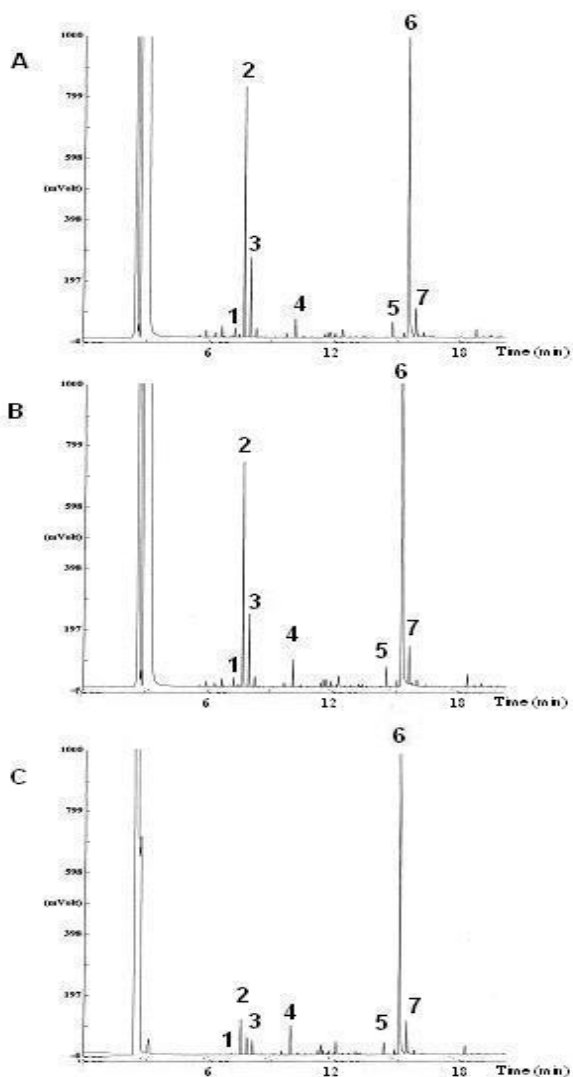


Figure 4. Gas chromatograms of essential oil of *Thymi herba* (A), *Species thymi composita* (B) and tea of *Species thymi composita* (C)

1.  $\beta$ -myrcene; 2. p-cymene; 3.  $\gamma$ -terpinene; 4. linalool; 5. terpinene-4-ol; 6. thymol; 7. carvacrol

*Althaeae folium* is rich in Ca (>30000 mg/kg), S (>5000 mg/kg) and Sr (>100 mg/kg), *Thymi herba* contains Mo and Ni over average plant concentration (3 and 10 mg/kg, respectively), while Sr concentration of *STC* is also salient<sup>22-23</sup>. All plant materials contain K in relatively low concentration (<20000 mg/kg). The poorest herb in mineral elements is *Liquiritiae radix* in which beside K the concentration of Mn, P, S and Zn also goes under the average plant concentration (<20 mg/kg, <2000 mg/kg, <20 mg/kg, respectively). The mineral element composition of *Althaeae radix* is similar to *Liquiritiae radix*.

The element concentration of most of the toxic and harmful elements in the teas is under the detection limit (Table 7.) which is favourable. According to our previous experiments and papers we can not see any significant concentration in the extracts<sup>20-23</sup>. The only relevant thing is that the solvability of Ca seems to be higher in *STC* which could be happened by the synergic effect of organic components in the drug mixture.

### 3.6. In vitro transfer of elements

The effect of *STC* may be connected to the organic bioactive agents but the examination of effectiveness and transfer of compounds is rather difficult. The flavonoid content in tea mixture by usual way was unable to determine because of the disturbing effect of mucilage. Therefore, the transfer of flavonoids is also not measurable and the examination of *in vitro* absorption of mucilage does not make sense because of its properties. According to our previous measurements the transfer of essential oils and volatile compounds provides only qualitative results. Since elements presenting in the extracts may also have favourable effect and they could be well measurable quantitative, the transfer of elements was determined.

Table 8. Amount of elements ( $\mu\text{g}$ ) transferred from 100 mL aqueous extract of *Species thymi composita* into buffer solution pH=1.1 and from buffer solution (pH=6.5) with extract of *Species thymi composita* into buffer solution pH=7.5

	30 min.	60 min.	90 min.	120 min.
<b>Into pH=1.1</b>				
Al	20.1	21.1	25.2	26.1
Ba	1.4	2.4	2.6	2.5
Ca	1301	1446	1542	1568
Cu	2.2	2.5	2.6	2.7
Fe	10.1	11.3	11.6	12.1
Mg	447	598	703	727
Mn	2.6	3.3	3.6	3.7
Ni	0.31	0.41	0.42	0.44
P	325	409	456	471
S	394	600	702	714
Sr	7.2	8.1	8.6	8.7
Zn	2.5	26	2.9	3.1
<b>Into pH=7.5</b>				
Al	3.0	3.4	5.2	6.6
Ba	0.4	0.6	0.8	0.9
Ca	391	443	505	618
Cu	1.7	2.0	2.3	2.4
Fe	3.1	3.7	3.9	4.4
Mg	288	446	562	591
Mn	0.8	1.1	1.5	1.6
S	258	405	537	573
Sr	4.2	4.5	5.0	6.0
Zn	0.82	0.89	0.90	0.91

The transfer of elements from tea and buffer solution pH=6.5 of *STC* into different buffer solutions (pH=1.1 and pH=7.5) was measured in a dialysis system. The result was evaluable for Al, Ba, Ca, Cu, Fe, Mg, Mn, S, Sr and Zn, while the concentration of As, Cd, Co, Cr, Hg, Mo, Pb, Se, V was under the detection limit. The transfer was continuous in all cases (Table 8). Relative high transfer was observed for Al, Ba and Mg in case of buffer solution pH=1.1 (stomach pH), since more than half of the amount in tea passed through the membran (Table 5). The transfer of Ni was obtained to be very low. From buffer solution pH=6.5 (intestine pH) to buffer solution pH=7.5 (plasma pH) the element transfer was found to be of lower rate and the amount of Mg passed from the extract of *STC* was only relevant with transfer of 591 mg (70% of the initial amount).

Table 6. Element content of drug components and *Species thymi composita* (mg/kg dry weight, n=3)

Elements	<i>Althaeae folium</i>	<i>Althaeae radix</i>	<i>Verbasci flos</i>	<i>Thymi herba</i>	<i>Liquiritiae radix</i>	<i>Species thymi composita</i>
Al	376.6±2.9	83.9±1.54	1168±7	641.7±9.8	47.7±0.3	961.4±20.6
As	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Ba	13.35±0.03	7.26±0.21	12.05±0.02	74.67±1.32	13.14±0.11	30.50±0.54
Ca	34044±267	6113±20	6616±31	12687±290	9709±63	17480±346
Cd	0.34±0.02	0.25±0.01	0.09±0.01	0.21±0.01	<0.03	0.14±0.01
Co	<0.015	<0.015	0.56±0.01	<0.015	<0.015	0.33±0.04
Cr	1.28±0.03	0.97±0.04	3.34±0.05	11.51±0.24	0.90±0.03	3.95±0.07
Cu	15.93±0.02	14.47±0.10	14.88±0.09	12.83±0.23	5.90±0.02	17.59±0.31
Fe	404.2±2.5	66.9±0.35	1288±6	563.5±9.4	69.0±0.2	878.5±13.2
Hg	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75
K	17331±79	11343±95	11509±58	15681±255	3946±45	13649±282
Li	5.95±0.46	<0.1	5.98±0.81	4.37±0.46	0.87±0.15	5.86±0.45
Mg	1679±11	1429±8	1758±3	1947±11	1441±11	1833±24
Mn	38.64±0.14	9.70±0.43	46.73±0.23	129.4±2.5	9.19±0.07	54.97±0.99
Mo	2.12±0.30	1.55±0.09	1.57±0.36	12.15±0.43	1.31±0.11	1.92±0.24
Na	883.8±5.01	1006±10	417.1±3	171.2±3.7	436.2±7.2	622.0±15.1
Ni	4.52±0.07	4.63±0.08	7.17±0.09	44.78±0.75	4.29±0.13	6.86±0.14
P	4115±11	3657±23	2946±15	1888±24	1146±8	3220±38
Pb	3.73±0.21	1.29±0.14	1.47±0.06	3.22±0.07	<0.5	1.39±0.07
S	6167±9	3506±7	1454±16	1853±19	876.8±3	3420±17
Se	<0.2	0.364 ±0.086	1.26±0.09	<0.2	<0.2	<0.2
Sr	108.6±0.77	61.29±0.49	35.36±0.27	40.45±0.73	334.5±5.2	146.8±3.4
V	1.01±0.14	<0.05	3.45±0.04	1.24±0.09	0.12±0.01	2.23±0.17
Zn	36.43±1.06	23.19±0.82	26.39±0.23	33.17±1.16	10.14±0.12	26.23±0.62

Table 7. Element content in teas of drug constituents and *Species thymi composita* (µg/100 mL, n=3)

Elements	<i>Althaeae folium</i>	<i>Althaeae radix</i>	<i>Verbasci flos</i>	<i>Thymi herba</i>	<i>Liquiritiae radix</i>	<i>Species thymi composita</i>
Al	0.37±0.04	0.92±0.02	158.0±0.9	30.61±0.93	10.14±0.10	26.98±0.36
As	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Ba	0.19±0.01	0.38±0.03	2.02±0.02	10.02±0.04	1.31±0.03	3.94±0.07
Ca	2427±33	949.2±3.6	1901±24	3137±11	514.3±4.3	3706±11
Cd	<0.01	<0.01	0.03±0.01	<0.01	<0.01	<0.01
Co	<0.015	<0.015	0.23±0.01	0.09±0.01	<0.015	0.17±0.02
Cr	0.43±0.02	0.64±0.02	2.06±0.03	2.05±0.02	0.68±0.01	1.86±0.01
Cu	0.69±0.02	2.39±0.05	7.60±0.11	3.35±0.01	3.02±0.02	6.78±0.02
Fe	1.07±0.13	2.52±1.89	175.2±1.9	32.7±0.25	8.48±0.03	30.33±0.09
Hg	<0.75	<0.75	<0.75	<0.75	<0.75	<0.75
K	11847±25	5627±34	8796±48	11109±71	2552±18	9104±34
Li	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Mg	576.2±5.5	920.3±4.8	886.0±5.3	992.1±6.6	937.0±2.2	838.3±0.9
Mn	0.79±0.05	0.53±0.16	11.94±0.18	35.70±0.13	2.35±0.02	11.53±0.02
Mo	1.55±0.02	1.17±0.11	1.33±0.11	1.69±0.05	1.16±0.08	1.49±0.08
Na	478.8±0.7	515.8±0.12	398.4±1.97	160.7±1.7	394.7±5.5	459.9±1.0
Ni	4.27±0.07	4.44±0.08	6.02±0.10	9.47±0.03	4.24±0.10	6.19±0.27
P	575.2±5.9	940.4±5.0	2446±30	1279±42	618.5±6.3	1978±14
Pb	<0.05	<0.05	0.58±0.09	0.54±0.02	<0.125	<0.125
S	2655±9	3299±15	976.8±10.6	1181±4	524.5±8.6	2480±11
Se	<0.2	<0.2	0.408±0.052	<0.2	<0.2	<0.2
Sr	7.49±0.35	8.42±0.09	7.12±0.12	6.71±0.52	27.08±0.31	19.09±0.28
V	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Zn	0.69±0.08	0.725±0.180	13.72±0.10	8.20±0.38	3.27±0.15	8.22±0.03

#### 4. Discussion and Conclusions

A tea mixture, *Species thymi composita*, known from the FoNo used in respiratory diseases was studied for possible analytical methods (morphological study and composition analyses) and the effectiveness was examined in vitro for elements.

The main macro- and micromorphological characteristics of STC belong to the drug components of mixture, which are well recognizable. The applicable analytical procedures for determination of bioactive agents and compositions were essential oil content and composition by GC and GC-MS, mucilage content, element content by ICP.

According to our analysis of *Thymus vulgaris* oil, the main bioactive compounds are thymol, p-cymene,  $\gamma$ -terpinene, carvacrol and linalool as was found by other authors as well<sup>24,25</sup>. Its volatile oil components with polyphenolic compounds have relevant antioxidant activities and spasmolytic effect which prove the effectiveness<sup>26,27</sup>. The mucilage containing *Althaea officinalis* extract has antitussive activity and inhibits the endothelin-1 (ET-1) induced mitogen activated protein kinase (MAPK) activation and calcium mobilization as well as cough caused by ACE drugs<sup>13,27,28</sup>. Extract of *Verbascum flos* has anti-inflammatory activity<sup>29</sup>.

Airway inflammation has a key role in the pulmonary and respiratory diseases, as well as in asthma. In these inflammatory processes inflammatory mediators, prostaglandins (PGD2, PGE2), leukotriens and cytokines such as IL-1 $\beta$ , IL-3, IL-5, IL-6, IL-8, IL-13, TNF- $\alpha$ , form by neutrophils, macrophages, lymphocytes, eosinophils and mast cells<sup>30-32</sup>. At transcriptional level the processes are regulated by NF- $\kappa$ B. The severity of inflammation is in relation to the elevated activity of NF- $\kappa$ B.

Prostaglandins (PGs) have a role in cough induced by angiotensin-converting enzyme (ACE) inhibitors<sup>33</sup>. Inhibition of PG synthesis by calcium channel blockers can reduce this effect<sup>34</sup>. Non-steroidal antiinflammatory drugs (NSAID-s) inhibit the biosynthesis of prostaglandins, and the synthesis of iNOS proinflammatory protein as well as proinflammatory cytokines, e.g. *Glycyrrhiza glabra* proved to have antioxidant activity and reduces inflammatory processes, ACE enzyme activity, PGE2 production, as well as LPS (lipopolysaccharide) induced TNF $\alpha$  and IL-1 $\beta$ , IL-6, mRNA expression<sup>5,35</sup>.

In PG synthesis reactive oxygen species formed inhibit the function of cyclooxygenase. The inhibition of COX2 is favourable, while of COX1 is contraindicated<sup>36</sup>. Several nutritional polyphenols and others are COX2 inhibitors and iNOS activators, therefore their uses, e.g. in form of tea, are beneficial<sup>37</sup>. Mainly the flavonoids in *Althaeae folium*, *Thymi herba* and *Verbasci flos* of STC may participate in this favorable effect.

In living organism transition metal ions (Cu, Fe, Mn, Zn) have a key function in both catalysis of biological oxidation by formation of free radicals and antioxidant

system by scavenging free radicals. The alteration of these element concentrations in the cells significantly modifies the signal transduction processes<sup>37,38</sup>. Metal ions participate in the activation of NF- $\kappa$ B, AP-1 and the regulation of I $\kappa$ B kinases and other redox systems<sup>39</sup>. Magnesium deficiency may induce transient increase in intracellular Ca level, in which process prooxidant cytokines (IL-1, IL-6, IL-8, TNF- $\alpha$ , - $\beta$ ), different growth factors (EGF- $\alpha$ , TGF- $\beta$ , NFGF, FGF, PDGF), and interferons (IFN- $\alpha$ , - $\gamma$ ) form. The elevated cytokine production induces genes of reactive oxygen species and produces the following enzymes: NADPH oxidase, xanthine oxidase/dehydrogenase, COX, lipoxygenase, Cyt P450, NO synthase, proteins containing iron, Cu,ZnSOD, MnSOD. At the same time increasing of the intracellular Mg level inhibits the production of prooxidant cytokines by activation of corresponding protein phosphatases<sup>40</sup>. The Mg absorption from the tea of STC seems to be granted, since the initial Ca to Mg concentration ratio was 4 in the tea, the ratio decreased to 2 in gastric acid, while in the plasma the ratio decreased further to 1 (Table 5). Meanwhile the normal plasma Ca to Mg concentration ratio is about 4 or 5, the absorption of tea with ratio of 1 indicates a higher Mg absorption that may affect indirectly against the mentioned proinflammatory process. The favourable effect of oral Mg administration in chronic obstructive pulmonary disease confirms this statement<sup>41</sup>. Since Mg as well as Zn have bronchodilatory action and antioxidant flavonoids and Se, Zn, Mn, Cu have relevant effect on respiratory diseases<sup>41,42</sup>, the presence and absorption of these elements in the tea may prove the effect.

The paper summarized the analytical methods that may use in the analysis of and characterize the tea mixture STC. In the development of favourable effect of STC, which acts via signal transduction process, the presence of bioactive flavonoids, volatile oil components, mucilage and elements may be jointly important.

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