

ANTIOXIDANT VALUE AND ELEMENT CONTENT IN SOME TINCTURES USED IN MEDICATION

KLÁRA SZENTMIHÁLYI,^{1*} ILONA SZÖLLŐSI VARGA,² ANITA GERGELY,³
MÁRIA RÁBAI¹ and MÁRIA THEN⁴

¹Institute of Materials and Environmental Chemistry, Research Centre of Natural Sciences,
Hungarian Academy of Sciences, H-1519 Budapest, P.O. Box 286, Hungary

²Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

³Department of Applied Chemistry, Corvinus University of Budapest,
H-1118 Budapest, P.O. Box 53, Hungary

⁴Institute of Pharmacognosy, Semmelweis University of Medicine, Budapest, Hungary

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Tinctures are almost the oldest medicines and their use is substantial in the medication nowadays as well. The antioxidant values by ferric reducing/antioxidant power (FRAP) method and element content by inductively coupled plasma optical emission spectrometry (ICP-OES) were investigated in some tinctures official in the VII. and VIII. Pharmacopoeia Hungarica. The highest FRAP values were found for volatile oil containing *Tinctura Aurantii amari epicarpium et mesocarpium*, *Tinctura Amara* and *Tinctura Valerianae* (764.54 ± 19.90 ; 757.37 ± 14.46 ; $826.40 \pm 5.89 \mu\text{mol l}^{-1}$, respectively). The correlations between the FRAP values and dilution with different alcohol content in *Tinctura Chinae*, *Tinctura Ipecacuanhae normata* and *Tinctura Strychni* were also investigated. Remarkable differences were found between the element concentrations in the different tinctures. The element contents in tinctures are not so high in absolute values nevertheless the presence of essential selenium, zinc, manganese and copper is important since they have key role in the antioxidant system. The common feature of the tinctures seems to be the lithium content. The Ca to Mg concentration ratio was found to be shifted towards magnesium in some of the tinctures that can show a higher Mg absorption which could affect against the proinflammatory processes in the cases of gastrointestinal diseases.

Keywords: Tincture – antioxidant activity – ferric reducing/antioxidant power – elements – ICP-OES – lithium

INTRODUCTION

Alcohol as a solvent is frequently used in pharmacy, perfumery and aroma industry [13]. Homeopathy also applies alcohol as a solvent for making mother tinctures to further dilution [5]. Tincture is the oldest medicine form nevertheless pharmaceutical science makes aqueous-alcoholic solutions with different alcohol content for the preferable gaining of bioactive agents. These extracts are generally prepared by maceration according to the description of *Pharmacopoeia Hungarica* [20, 21]. A part of tinctures is missing from the VIII. *Pharmacopoeia Hungarica* although their use is still substantial in the daily practice. Tinctures have more kinds of effects although the main bioactive agents are volatile oils or tannins or both.

*Corresponding author; e-mail address: szentmihalyi.klara@ttk.mta.hu

The Tinctura Amara, Tinctura Aromatica, Tinctura Aurantii and Tinctura Strychni have appetizing and peptic effects. Tinctura Chinae has cholagogue and fever reducer effects, Tinctura Chamomilae and Tinctura Thymi have antiseptic and antiinflammatory effects, Tinctura Ratanhiae has astringent, Tinctura Ipecacuanhae normata and Tinctura Saponariae have expectorant effects, and Tinctura Valerianae has sedative effect [6, 12, 24, 27].

At the standardization of the tinctures some kind of features (color, odor, taste, density) and bioactive agent contents of the extracts have to be determined. There are other components in the tinctures, for example mineral elements for that there were no measurements so far. At the same time the antioxidant properties of only some tinctures have been determined so far which are commercially available and official in other pharmacopoeias [10, 16, 19]. Tinctura Chamomilae and Tinctura Valerianae used in homeopathy and alcoholic extract of thyme as a spice were also investigated nevertheless by different methods [29, 35]. Most of the tinctures investigated for antioxidant activities and published in the literature are not used for medication in Hungary and are not official in the Pharmacopoeia Hungarica, e.g. tinctures made from *Ginkgo biloba*, *Panax ginseng*, *Pedilanthus titymaloides*, *Taxus baccata*, *Fagus sylvatica*, *Chionanthus virginicus*, *Baccharis incarum* [1, 8, 14, 18, 22, 35].

Tinctures are able to absorb relatively fast since they can transfer directly via mucous membrane and there is no need to pass through all digestive system. Therefore active agents and elements in tinctures affect relatively quickly in spite of their small amount [4]. Polyphenolic active agents, as flavonoids, tannins, have antioxidant activities [30] and their presence in the extracts, e.g. hesperidin in Tinctura Aurantii amari epicarpium et mesocarpium or tannins in Tinctura Ratanhiae, may be favourable in view of antioxidative value. Since volatile oils have relevant antioxidant activity it is supposed that the volatile oil containing tinctures also have antioxidant activities [4]. At the same time some trace elements, e.g. Fe, Mn, Cu have a key role in the biological oxidation and the antioxidant defence system as well and the presence of these elements in the tinctures can modify the antioxidant activities of the extracts [31].

Therefore some medicative tinctures available in the VII. and VIII. Pharmacopoeia Hungarica were investigated for their antioxidant value by ferric reducing/antioxidant power (FRAP) method and element content by inductively coupled plasma optical emission spectrometry (ICP-OES).

MATERIALS AND METHODS

Materials

The following tinctures official in the VII. and VIII. Pharmacopoeia Hungarica [20, 21] were examined: Tinctura Amara, Tinctura Aromatica, Tinctura Aurantii amari epicarpium et mesocarpium, Tinctura Chamomilae, Tinctura Chinae, Tinctura Ipecacuanhae normata, Tinctura Ratanhiae, Tinctura Saponariae, Tinctura Strychni, Tinctura Thymi, Tinctura Valerianae.

Preparation of tinctures by traditional method

Tinctura Amara, Tinctura Aromatica, Tinctura Aurantii amari epicarpium et mesocarpium, Tinctura Chamomilae, Tinctura Thymi, Tinctura Chinae, Tinctura Ipecacuanhae normata, Tinctura Saponariae and Tinctura Strychni were made according to the VII. Pharmacopoeia Hungarica [20], while the preparation of Tinctura Ipecacuanhae normata, Tinctura Ratanhiae and Tinctura Valerianae was done by the description of the VIII. Pharmacopoeia Hungarica [21].

Water was used as a solvent for determination of reducing power in diluted solutions of different tinctures. The dilution rates were 2×, 5× and 10×.

Official test methods

For controlling and measuring the bioactive agents in the tinctures the description of Pharmacopoeia Hungarica [20, 21] were followed. The results were suited to the requirements.

Measurements of antioxidant values

For measurement of the ferric reducing/antioxidant power (FRAP), the following FRAP reagent solution was used according to a method modified for plant materials by Szöllösi and Szöllösi Varga: 25 ml acetate buffer (300 mmol l⁻¹, pH = 3.6; 3.1 g CH₃COONa · 3H₂O and 16 ml glacial acetic acid in 1000 ml buffer solution), 2.5 ml TPTZ solution (10 mmol l⁻¹ 2,4,6-tripyridyl-S-triazine in 40 mmol l⁻¹ HCl) and 2.5 ml FeCl₃ · 6H₂O solution (20 mmol l⁻¹ FeCl₃ · 6H₂O in distilled water). For sample measurement 50 µl sample solution was added to the 1.5 ml FRAP reagent. The blank was a FRAP reagent. Absorbance was determined spectrometrically at 593 nm with 1 cm light path at 37 °C. Calculations were based on the calibration curve of known concentrations of Fe²⁺ ions using FeSO₄ · 7H₂O [2, 11, 33].

Measurement of element content by ICP-OES

Tincture of 25 ml was evaporated to dry then the residue was digested with 10 ml HNO₃ of 67% and 5 ml H₂O₂ of 30%. After digestion the sample was filled to 25 ml with bidistilled water from which concentration of elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sn, Sr, Ti, V, Zn) was determined with Spectro Genesis ICP-OES (ICP-OES, Kleve, Germany) equipment [29]. The concentrations of As, B, Cd, Hg, Ti and V were below the detection limits therefore these elements were omitted from the table.

Measurement of selenium by voltammetry

Selenium content of the samples was determined with a hanging mercury drop electrode by a cathodic stripping voltammetric method (instrument: Trace Lab 50, Radiometer, Copenhagen). The digested samples (2 ml) for ICP-OES were used in this measurement with 2 ml bidistilled water and 2 ml 1 M HCl as supporting electrolyte. The preconcentration was performed at -350 mV. Electrolysis time: 100 sec, potential: from -300 mV up to -900 mV, step duration -50 mV/s [17].

RESULTS

Antioxidant activity of tinctures

For determination of antioxidant activity of biological systems the FRAP (ferric reducing/antioxidant power) method is frequently used therefore it has been revealed that whether this method is suitable for measurement of antioxidant activities of tinctures [9]. The FRAP values of the tinctures are summarized in Table 1. Significant differences were found between the FRAP values of different tinctures. The highest values were found for volatile oil containing Tinctura Aurantii amari epicarpium et mesocarpium, Tinctura Amara and Tinctura Valerianae although Tinctura Chamomilae and Tinctura Saponariae have also remarkable reducing ability. The volatile oil containing Tinctura Aromatica and the tannin containing Tinctura Ratanhiae are without reducing ability and Tinctura Thymi has the least FRAP value. The other tinctures showed smaller reducing ability.

Table 1
FRAP (ferric reducing/antioxidant power) values ($\mu\text{mol l}^{-1}$) of tinctures

Tincture	FRAP value ($\mu\text{mol l}^{-1}$)
Tinctura Amara	757.37 \pm 14.46
Tinctura Aromatica	–
Tinctura Aurantii amari epicarpium et mesocarpium	764.54 \pm 19.90
Tinctura Chamomilae	654.34 \pm 10.02
Tinctura Chinae	277.82 \pm 3.93
Tinctura Ipecacuanhae normata	452.50 \pm 26.55
Tinctura Ratanhiae	–
Tinctura Saponariae	684.75 \pm 10.02
Tinctura Strychni	470.17 \pm 11.14
Tinctura Thymi	175.76 \pm 4.28
Tinctura Valerianae	826.40 \pm 5.89

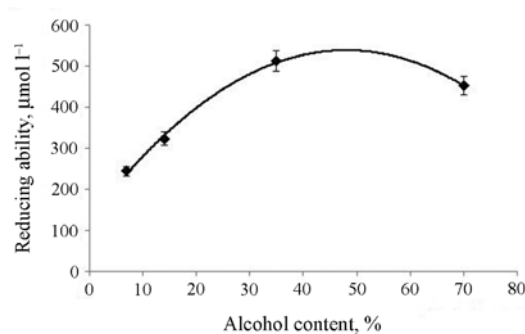


Fig. 1. Change of FRAP values ($\mu\text{mol l}^{-1}$) in Tinctura Ipecacuanhae normata by dilution in the function of alcohol concentration (the dilution was made from the 70% tincture with water)

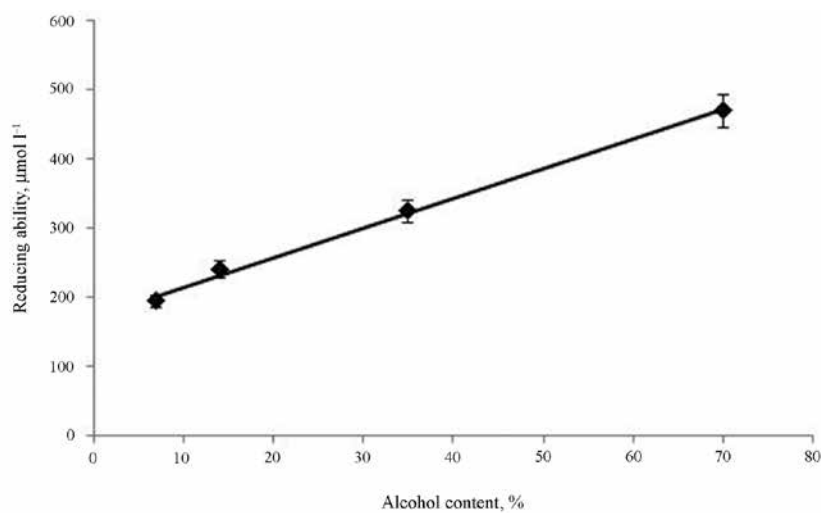


Fig. 2. Change of FRAP values ($\mu\text{mol l}^{-1}$) in Tinctura Strychni by dilution in the function of alcohol concentration (the dilution was made from the 70% tincture with water)

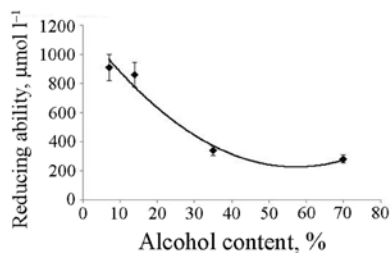


Fig. 3. Change of FRAP values ($\mu\text{mol l}^{-1}$) in Tinctura Chinae by dilution in the function of alcohol concentration (the dilution was made from the 70% tincture with water)

Table 2
Element content ($\mu\text{g ml}^{-1}$) in tinctures

	Tinctura Amara	Tinctura Aromatica	Tinctura Aurantii amari epicarpium et mesocarpium	Tinctura Chamomilae	Tinctura Chinae	Tinctura Ipecacuanhae normata
($\mu\text{g ml}^{-1}$)						
Al	1.441 \pm 0.014	1.034 \pm 0.023	1.415 \pm 0.006	1.053 \pm 0.13	1.579 \pm 0.005	0.758 \pm 0.009
Ba	0.266 \pm 0.002	0.217 \pm 0.006	0.261 \pm 0.001	0.215 \pm 0.004	0.376 \pm 0.002	0.340 \pm 0.008
Ca	91.44 \pm 1.79	1.275 \pm 0.159	58.22 \pm 0.44	17.26 \pm 1.49	44.04 \pm 0.68	0.606 \pm 0.077
Co	0.041 \pm 0.001	0.036 \pm 0.001	0.034 \pm 0.001	0.042 \pm 0.011	0.042 \pm 0.001	0.035 \pm 0.001
Cr	0.038 \pm 0.001	0.035 \pm 0.001	0.049 \pm 0.001	0.236 \pm 0.007	<0.004	<0.004
Cu	0.385 \pm 0.004	0.117 \pm 0.001	0.109 \pm 0.001	0.719 \pm 0.014	0.250 \pm 0.001	0.041 \pm 0.001
Fe	0.484 \pm 0.007	0.301 \pm 0.009	0.995 \pm 0.002	0.744 \pm 0.037	1.795 \pm 0.006	0.172 \pm 0.007
K	134.5 \pm 1.0	63.88 \pm 0.34	88.74 \pm 0.29	148.7 \pm 13.9	51.04 \pm 0.09	0.738 \pm 0.028
Li	0.112 \pm 0.002	0.061 \pm 0.002	0.164 \pm 0.006	0.043 \pm 0.006	0.143 \pm 0.008	0.026 \pm 0.001
Mg	38.10 \pm 0.47	25.30 \pm 0.31	40.55 \pm 0.01	65.31 \pm 3.78	41.29 \pm 0.05	1.051 \pm 0.030
Mn	2.748 \pm 0.031	0.456 \pm 0.009	0.253 \pm 0.001	0.171 \pm 0.005	0.879 \pm 0.009	0.015 \pm 0.001
Mo	0.007 \pm 0.005	0.013 \pm 0.001	0.014 \pm 0.003	0.024 \pm 0.008	0.033 \pm 0.008	0.0058 \pm 0.0012
Na	25.91 \pm 0.44	29.24 \pm 0.09	28.44 \pm 0.14	156.8 \pm 16.9	17.95 \pm 0.05	1.829 \pm 0.120
Ni	0.174 \pm 0.001	0.072 \pm 0.002	0.127 \pm 0.001	0.318 \pm 0.010	0.213 \pm 0.001	0.027 \pm 0.001
P	32.99 \pm 0.40	9.016 \pm 0.102	14.85 \pm 0.09	115.5 \pm 9.4	9.904 \pm 0.052	0.177 \pm 0.018
Pb	0.033 \pm 0.002	0.034 \pm 0.001	0.062 \pm 0.002	0.045 \pm 0.002	0.036 \pm 0.001	0.038 \pm 0.004
S	1660 \pm 22	645.0 \pm 15.4	1087 \pm 1	1457 \pm 23	345.2 \pm 1.9	24.89 \pm 0.46
Se*	0.00322	0.00103	0.00056	<0.0002	0.00235	<0.0002
Si	5.377 \pm 0.038	1.294 \pm 0.067	3.862 \pm 0.280	2.759 \pm 0.121	3.022 \pm 0.037	0.075 \pm 0.032
Sn	0.230 \pm 0.005	0.194 \pm 0.007	0.218 \pm 0.002	0.156 \pm 0.002	0.191 \pm 0.006	0.292 \pm 0.009
Sr	0.171 \pm 0.021	0.034 \pm 0.001	0.869 \pm 0.003	0.058 \pm 0.001	0.166 \pm 0.001	0.064 \pm 0.001
Zn	5.289 \pm 0.065	2.806 \pm 0.053	5.227 \pm 0.007	1.340 \pm 0.035	2.109 \pm 0.011	0.104 \pm 0.005

*One measurement.

In homeopathic curing there are frequently used 98% alcoholic extracts and their dilutions were made from alkaloid containing drugs therefore we were interested in the changes of reducing power by the dilution. The reducing ability of tinctures with the dilution is very different according to tincture investigated. The result of Tinctura Ipecacuanhae normata is shown in Fig. 1. First the reducing power increased by the dilution to the double volume then decreased for further dilutions. The reducing ability of Tinctura Strychni shows a linear decrease with the dilution (Fig. 2), while in the case of Tinctura Chinae the FRAP value increased with the dilution (Fig. 3). The explanation of this very large scale difference of behavior is that the dilution of tinc-

Table 2 (cont.)

	Tinctura Ratanhiae	Tinctura Saponariae	Tinctura Strychni	Tinctura Thymi	Tinctura Valerianae
($\mu\text{g ml}^{-1}$)					
Al	1.073 \pm 0.006	0.716 \pm 0.025	0.976 \pm 0.004	1.028 \pm 0.004	0.779 \pm 0.003
Ba	0.028 \pm 0.002	0.003 \pm 0.007	0.308 \pm 0.001	0.416 \pm 0.001	0.270 \pm 0.004
Ca	2.923 \pm 0.009	22.15 \pm 4.40	1.485 \pm 0.014	786.0 \pm 11.2	0.457 \pm 0.004
Co	0.034 \pm 0.001	0.010 \pm 0.001	0.045 \pm 0.001	0.052 \pm 0.01	0.043 \pm 0.001
Cr	<0.004	<0.004	<0.004	0.031 \pm 0.001	0.078 \pm 0.001
Cu	0.078 \pm 0.001	0.351 \pm 0.023	0.188 \pm 0.001	0.186 \pm 0.002	0.154 \pm 0.001
Fe	1.390 \pm 0.002	1.020 \pm 0.130	0.538 \pm 0.001	0.200 \pm 0.002	0.094 \pm 0.001
K	20.22 \pm 0.13	781.1 \pm 12.0	35.93 \pm 0.04	157.1 \pm 0.9	78.16 \pm 0.87
Li	0.048 \pm 0.003	0.022 \pm 0.001	5.765 \pm 0.01	0.175 \pm 0.009	0.128 \pm 0.029
Mg	9.738 \pm 0.035	32.30 \pm 0.14	27.41 \pm 0.15	19.50 \pm 0.04	30.34 \pm 0.45
Mn	0.015 \pm 0.001	0.071 \pm 0.011	2.036 \pm 0.007	2.138 \pm 0.023	0.144 \pm 0.008
Mo	0.049 \pm 0.001	0.041 \pm 0.001	0.026 \pm 0.003	0.012 \pm 0.001	0.071 \pm 0.006
Na	11.27 \pm 0.06	16.76 \pm 1.75	21.26 \pm 0.09	28.28 \pm 0.003	24.13 \pm 0.31
Ni	0.085 \pm 0.001	0.209 \pm 0.001	3.318 \pm 0.008	0.320 \pm 0.005	0.397 \pm 0.013
P	0.929 \pm 0.010	90.14 \pm 12.08	3.334 \pm 0.062	29.31 \pm 0.35	14.02 \pm 0.05
Pb	0.041 \pm 0.004	<0.003	0.037 \pm 0.002	0.028 \pm 0.004	0.028 \pm 0.004
S	148.8 \pm 0.3	<1	449.6 \pm 11.5	4486 \pm 37	438.0 \pm 11.0
Se*	<0.0002	<0.0002	0.00253	0.00123	0.00044
Si	3.685 \pm 0.168	3.048 \pm 0.23	1.155 \pm 0.037	12.60 \pm 0.30	3.53 \pm 0.06
Sn	0.257 \pm 0.001	<0.01	0.154 \pm 0.002	0.172 \pm 0.008	0.300 \pm 0.006
Sr	0.025 \pm 0.001	0.014 \pm 0.004	0.022 \pm 0.001	0.930 \pm 0.013	0.013 \pm 0.001
Zn	0.0279 \pm 0.001	1.149 \pm 0.059	6.212 \pm 0.031	1.378 \pm 0.009	0.666 \pm 0.007

*One measurement.

ture with water may cause hydrolysis of some compounds and precipitation of some active components and depending on the properties of these components (antioxidant or prooxidant) the antioxidant power alters.

These results involve the conclusion, that every kind of tincture may have their maximal FRAP value at different dilution unit, so the comparative results in Table 1. concern only the tinctures official in the Pharmacopoeia Hungaricas without any dilution. Each tincture should be measured by different dilutions to get to know the maximal reducing ability in aim to carry out a priority.

Element content in tinctures

The element content in the alkaloid containing tinctures (*Tinctura Chinae*, *Tinctura Ipecacuanhae* and *Tinctura Strychni*) is shown in Table 2. Although the element contents are relatively low, remarkable differences can be seen between the element concentrations in the different tinctures. The highest element contents can be found in *Tinctura Chinae* in aspect of most elements, but Li, Mn, Ni, S and Zn contents reach their maximal amount in *Tinctura Strychni*.

The element contents in volatile oil containing tinctures (*Tinctura Amara*, *Tinctura Aromatica*, *Tinctura Aurantii amari epicarpium et mesocarpium*, *Tinctura Chamomilae*, *Tinctura Thymi*, *Tinctura Valerianae*) are significantly different (Table 2). The common feature of these tinctures is that all of them contain lithium and *Tinctura Thymi* and *Tinctura Aurantii amari epicarpium et mesocarpium* were found to be the best sources of lithium. *Tinctura Chamomilae* contains the highest concentration of Cu, Mg and P. The best K concentration can be found in *Tinctura Thymi*, which is also the richest source for S and Mn beside *Tinctura Amara*. Zn is contained in an elevated amount in *Tinctura Amara* and *Tinctura Aurantii amari epicarpium et mesocarpium*.

The element content of tannin containing *Tinctura Ratanhiae* which is mainly externally used for painting and triterpene saponine containing *Tinctura Saponariae* are presented in Table 2 as well. There was not found any relevant element concentration in the tinctures. *Tinctura Saponariae* is by far richer in Ca, Mg, K and P content than *Tinctura Ratanhiae*. There can be multiple amounts discovered in *Tinctura Saponariae*.

DISCUSSION

Tinctures used in medication have significance nowadays as well. Some of them can be featured with relevant reducing ability measured by FRAP method that can be important in the development of effect and furthermore a substantial data for the valuation of tinctures [9]. The reducing ability of tinctures changed differently with the dilution according to tincture investigated. In homeopathic relation the most promising alteration was shown by *Tinctura Chinae* since the reducing ability increased with the dilution, while it was getting higher with bigger alcohol content in *Strychni*. *Tinctura Ipecacuanhae normata* has a maximal antioxidant power at 45–50% of alcohol content. The reducing abilities of tinctures are complex effect of antioxidant (e.g. flavonoids, tannins) and prooxidant agents (alcohol, iron and other transition metal ions) in the extracts [25, 31].

The element contents in tinctures are not so high in absolute values nevertheless the presence of essential selenium, zinc, manganese and copper is important since they have key role in the antioxidant system [26]. The Ca/Mg concentration ratio is 5 : 1 in the blood, and it was found to be 1 : 2 in the expectorant *Tinctura Ipecacuanhae normata* and 1 : 20 in *Tinctura Strychni*. These shifted Ca/Mg concentration ratios from the normal rate in blood can show a higher Mg absorption which could affect

against the proinflammatory processes in the cases of gastrointestinal diseases [7, 32]. However Tinctura Chinae contains a smaller 1 : 1 ratio of Ca to Mg and the concentrations of these elements are significantly higher than in the other tinctures. The presence of essential elements and the favourable Ca to Mg concentration ratio in most of the alkaloid containing extracts surely contribute to the preferential action of the tinctures, mainly in the cases of the proinflammatory processes of gastrointestinal diseases.

Lithium content is a common feature of volatile oil containing tinctures which is known as a tranquillizer [23]. The Ca/Mg concentration ratios are generally smaller than the normal ratio in blood which can mean a favorable effect in the antiinflammatory processes. The exception from this is Tinctura Thymi, which has got a surprisingly enormous amount of Ca and therefore a respectively high Ca/Mg ratio.

The tannin containing expectorant Tinctura Saponariae has 2 : 3 Ca/Mg concentration ratio while the ratio is 1 : 3 in the Tinctura Ratanhiae, both of them are favorable in the aspect of treatment.

The tinctures investigated, except of Tinctura Valerianae, affect through gastrointestinal system as expectorant, appetizing, cholagogue, antiseptic and antiinflammatory extract. During inflammatory process inflammatory mediators, prostaglandins (PGD₂, PGE₂), leucotrienes and cytokines (IL-1 β , -3, -6, -8, -13, TNF- α) form [3]. By prostaglandin synthesis reactive oxygen species arise that inhibit the function of cyclooxygenases (COX1, COX2). Inhibition of COX2 is favorable in the decreasing of inflammatory processes acting like Ca channel blockers as well [3, 31]. Therefore the shifted Ca/Mg concentration ratio from 5 : 1 to 1 : 1–1 : 20 in the tinctures takes effect against the inflamed process. At the same time these altered Ca/Mg concentration ratios inhibit the activation of PLC (phospholipase C), PKC (protein kinase C), MAPKKK (mitogen activated protein kinases) and therefore the activation of NF-kappaB (nuclear factor) as well which process acts also against the inflammation [32].

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