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## VARIATION OF CHEMICAL COMPOSITION OF MEDICINAL HERBS OF DIFFERENT PRODUCERS

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### Abstract

The paper goal was to measure the content of bioactive substances, such as hypericins, some flavonoids and hydroxycinnamic acids, and concentrations of chemical elements, including essential macro- (K, Mg, and Na), microelements (Zn, Mn, Cu, Fe, Cr, Ni, and Co), as well as toxic metals (Pb and Cd), in samples of St. John's wort herbs of four different producers. High-performance liquid chromatography and atomic absorption spectroscopy were used; sample preparation was based on the procedures described in the State Pharmacopoeia of Ukraine. Significant variation in the content of organic compounds was detected: the difference between the maximum and minimum concentrations reaches a factor of 5 for hypericins, 10 for rutin and 2 for hyperoside. The concentration of quercetin was more stable than those of other flavonoids, and its fluctuations did not exceed 30%. The measured concentrations of hypericin and hyperoside regulated by the Pharmacopoeia, were found to be lower than the norms. Elemental composition is also characterised by variability. Changes in the concentration of essential macroelements (K, Mg and Na) and some of the microelements (Zn, Cu and Ni) do not exceed 30-40%. The concentration of other essential microelements (Fe, Mn, Co and Cr) varies in much wider limits - from a factor of 2 (Mn) to 6 (Fe). In most cases, the measured concentrations of Pb and Cd fluctuate in the ranges of 0.15–0.25 µg/g and 0.25–0.35 µg/g, respectively. However, some samples are enriched to 0.7–0.75 µg/g with both toxic metals. The measured concentrations are lower than the WHO norm for Pb (10 µg/g) in all samples and exceed the norm for Cd (0.3 µg/g) in three out of four studied samples. Statistically significant positive or negative correlations were found between the concentrations of certain elements (K, Mg, Fe, Mn and Cd) and the content of organic substances (rutin, hyperoside, hypericin and rosemary and chlorogenic acids). From a practical viewpoint, the most important are positive correlations of Fe with rutin, as well as negative correlations between Mn and hyperoside, and Cd and chlorogenic acid. Existing requirements and norms for medicinal plants in Ukraine do not guarantee the stability of the chemical composition of plants that are sold in the pharmacy network and, accordingly, the stability of their therapeutic properties.

**Keywords:** Hypericum perforatum, herb, St. John's wort, elemental composition, hypericin, rutin, hyperoside, atomic absorption spectroscopy, high-performance liquid chromatography

## ВАРІАЦІЯ ХІМІЧНОГО СКЛАДУ ЛІКАРСЬКИХ ТРАВ РІЗНИХ ВИРОБНИКІВ

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### Анотація

Метою статті було визначити вміст біологічно активних речовин, таких як гіперіцини, деякі флавоноїди та гідроксикоричні кислоти, а також концентрації хімічних елементів, включаючи основні макро- (K, Mg, Na), мікроелементи (Zn, Mn, Cu, Fe, Cr, Ni, Co), а також токсичні метали (Pb і Cd) у зразках трави звіробою чотирьох різних виробників. Використано методи високоефективної рідинної хроматографії та атомної абсорбційної спектроскопії, пробопідготовка проводилася на основі процедур, описаних у Державній Фармакопеї України. Виявлено значну варіативність вмісту органічних сполук: різниця між максимальною та мінімальною концентраціями сягає в 5 разів для гіперіцинів, 10 для рутину та 2 для гіперозиду. Концентрація кверцетину була більш стійкою, ніж у інших флавоноїдів, а її коливання не перевищували 30%. Виміряні концентрації гіперіцину та гіперозиду, які регулюються Фармакопеею, виявилися нижчими за норми. Елементний склад також характеризується мінливістю. Зміни концентрації основних макроелементів (K, Mg, Na) та деяких мікроелементів (Zn, Cu, Ni) не перевищують 30-40%. Концентрація інших важливих мікроелементів (Fe, Mn, Co, Cr) варіюється у набагато більш широких межах – від коефіцієнту 2 (Mn) до 6 (Fe). У більшості випадків

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виміряні концентрації Pb та Cd коливаються в межах 0.15–0.25 мкг/г і 0.25–0.35 мкг/г, відповідно. Проте деякі зразки збагачені до 0.7–0.75 мкг/г за обома токсичними металами. Винайдені концентрації нижче норм ВООЗ для Pb (10 мкг/г) у всіх зразках та перевищують норму для Cd (0.3 мкг/г) у трьох з чотирьох досліджених зразків. Встановлено статистично значимі позитивні або негативні кореляції між концентраціями певних елементів (K, Mg, Fe, Mn, Cd) та вмістом органічних речовин (рутин, гіперозид, гіперичин, розмаринова та хлорогенова кислоти). З практичної точки зору найбільш важливими є позитивні кореляції у парі Ферум-рутин, а також негативні кореляції між Манганом і гіперозидом та Кадмієм та хлорогеновою кислотою. Існуючі вимоги та норми щодо лікарських рослин в Україні, що продаються в аптечній мережі, не гарантують стабільності хімічного складу рослини, та, відповідно, стабільності їх лікувальних властивостей.

**Ключові слова:** *Hypericum perforatum*, трави, звіробій, елементний склад, гіперичин, рутин, гіперозид, атомна абсорбційна спектроскопія, високоефективна рідинна хроматографія

## ВАРИАЦИЯ ХИМИЧЕСКОГО СОСТАВА ЛЕКАРСТВЕННЫХ ТРАВ РАЗНЫХ ПРОИЗВОДИТЕЛЕЙ

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### Аннотация

Целью статьи было определить содержание биологически активных веществ, таких как гиперичин, некоторые флавоноиды и гидроксикоричные кислоты, а также концентрации химических элементов, включая основные макро- (K, Mg, Na), микроэлементы (Zn, Mn, Cu, Fe, Cr, Ni, Co), а также токсичные металлы (Pb и Cd) в образцах травы зверобоя четырех различных производителей. Использованы методы высокоэффективной жидкостной хроматографии и атомной абсорбционной спектроскопии. Пробоподготовка проводилась на основе процедур, описанных в Государственной Фармакопее Украины. Обнаружена значительная вариативность содержания органических соединений: разница между максимальной и минимальной концентрациями достигает 5 раз для гиперичинов, 10 для рутина и 2 для гиперозида. Концентрация кверцетина была более устойчивой, чем других флавоноидов, а ее колебания не превышали 30%. Измеренные концентрации гиперичина и гиперозида, которые регулируются Фармакопеей, оказались ниже нормы. Элементный состав также характеризуется изменчивостью. Изменения концентрации основных макроэлементов (K, Mg, Na) и некоторых микроэлементов (Zn, Cu, Ni) не превышают 30–40%. Концентрация других важных микроэлементов (Fe, Mn, Co, Cr) варьируется в гораздо более широких пределах - от коэффициента 2 (Mn) до 6 (Fe). В большинстве случаев измеренные концентрации Pb и Cd колеблются в пределах 0.15–0.25 мкг/г и 0.25–0.35 мкг/г, соответственно. Однако некоторые образцы обогащены до 0.7–0.75 мкг/г по обоим токсичным металлам. Обнаруженные концентрации ниже норм ВООЗ для Pb (10 мкг/г) во всех образцах и превышают норму для Cd (0.3 мкг/г) в трех из четырех исследованных образцов. Установлены статистически значимые положительные или отрицательные корреляции между концентрациями определенных элементов (K, Mg, Fe, Mn, Cd) и содержанием органических веществ (рутин, гиперозид, гиперичин, розмариновая и хлорогеновая кислоты). С практической точки зрения наиболее важны положительные корреляции в паре Ферум-рутин, а также отрицательные корреляции между Манганом и гиперозидом, а также Кадмием и хлорогеновой кислотой. Существующие требования и нормы для лекарственных растений в Украине, которые продаются в аптечной сети, не гарантируют стабильности их химического состава и, соответственно, стабильности их лечебных свойств.

**Ключевые слова:** *Hypericum perforatum*, травы, зверобой, элементный состав, гиперичин, рутин, гиперозид, атомная абсорбционная спектроскопия, высокоэффективная жидкостная хроматография.

### Introduction

Saint John's wort (SJW) is one of the most widely distributed medicinal plants. Its therapeutic action is associated with the presence of at least seven groups of medicinally active compounds [1]. The most typical classes include phloroglucinols, flavonoids, naphthodianthrone, some organic acids and essential oils [2; 3]. Infusions of SJW are used as antiseptic agents in colitis; tinctures are applied for greasy gums and mouthwashes; there are known preparations on the basis of SJW with antibacterial, wound-healing, anti-inflammatory, vitamin and antidepressant actions [4–6].

SJW produces dozens of biologically active substances, but two compounds, namely hypericin

and hyperforin, are usually considered as the main active pharmaceutical ingredients (API) in SJW. Hypericin is an anthraquinone derivative, which belongs to naphthodianthrone and is naturally found in the yellow flower of SJW. This compound is characterised by antidepressant, potential antiviral, antineoplastic and immunostimulating activities.

Hyperforin (lipophilic phloroglucinol) may give even a stronger therapeutic effect for depression [5] compared to hypericin. It has only been found in the *Hypericum* genus of which *Hypericum perforatum* contains the highest concentrations. The compound has also shown antibacterial properties as well as anti-proliferate and pro-apoptotic effects towards some cancer

cells [6]. Adhyperforin, which differs from hyperforin by replacement of a methyl group in the isopropyl moiety with an ethyl group, produces a similar effect to hyperforin. In general, antidepressant activity was observed for phloroglucinol derivatives, naphthodianthrone and some flavonoids. The role and the mechanisms of these different compounds are still under discussion. However, the simplistic explanation, like one plant – one active ingredient – one mechanism is incorrect. The multiple bioactive compounds contribute to the therapeutic action of the plant in a complex manner [3]. In other words, some indications denote the synergistic activity of hypericins, hyperforins and flavonoids, which may explain why herbal preparations seem to have robust effectivity in spite of their variable composition.

Some other compounds, including the flavonoids rutin, quercetin and hyperoside, and hydroxycinnamic acids, such as neochlorogenic, chlorogenic, caffeic and rosemary acids, also appear to have physiological effects.

Rutin, hyperoside and quercetin present in many plant species. All these flavonoids are natural antioxidants [7]. Hyperoside has a greater reducing power than rutin because of possibly the smaller sugar group attached [8]. Quercetin has a higher reducing power than both rutin and hyperoside, probably due to the lack of a sugar group [8].

Rutin and quercetin act as metal chelators; they can bind Cu, Fe, Al, Zn and Mn [9–11]. Complexation of rutin to metal ions influences its antioxidant capacity. For example, the antioxidant capacity of rutin-Cu complexes increased eight-fold compared to rutin alone [10]. In contrast, the antioxidant activity of Fe-based complexes is much lower, in some cases approaching the activity of rutin [10]. Moreover, Fe-rutin complexes can also stimulate to some degree spontaneous oxygen radical production.

Chlorogenic acid and its isomer, neochlorogenic acid possess pronounced antioxidative properties and also exhibit antibacterial, antiviral, antipyretic and anti-obesity activities [12]. In fact, the antioxidant capacity of chlorogenic acid is higher than those of vitamin C and vitamin E. Caffeic acid is reported to have anticancer properties [13]; rosemary acid (an ester of caffeic acid) is considered as potential anxiolytic means.

At national levels, two fundamentally different approaches to the quality of herbal medicines exist in the world [14]. In many countries, for example, the EU, herbal medicines used for

diagnosis, treatment or prevention are considered as a medicinal agent, and all stages of their circulation are regulated by pharmaceutical legislation. An example of another approach is the practice in the United States, where herbal medicines are regulated by the relevant documents for food supplements. Herbal medicines often receive the status of dietary supplements which does not require pre-clinical research and clinical trials according to a pre-approved protocol.

In both approaches, the most important factor that determines the actual quality of herbal remedies is the quality of crude medicinal plants [15]. In accordance with international principles, the process of standardisation of medicinal plants should be based on the results of the research, referring to the three main pharmacopoeial definitions [16; 17] and should answer three questions regarding:

1) Identity – is the plant what it is to be?

2) Purity – are there any pollutions (botanical, microbiological or chemical contamination) and do they not exceed the specified limits?

3) Content – is there an API within the specified concentration limits?

Currently, there are no official generally recognised rigorous approaches to the creation of a quality assurance system in Ukraine. Such a system should cover all elements that influence the quality, efficacy and safety of herbal medicines from the cultivation of medicinal plants through drug development and to stages of production, quality control, storage and use. Nevertheless, some regulations refer to isolated stages of herbal medicine production, including cultivation, collection and processing of medicinal plants, and production storage, transportation and distribution of herbal medicines. All these documents are recommendatory; their hierarchy is described elsewhere [14; 18].

Those producers, who use their own approaches to quality assurance in their production, are usually based on their experience, scientific research data, and documents of a recommendatory nature. The analysis of compositions of herbal medicines, which are listed in the State Register of Medicinal Products of Ukraine, corroborates such a conclusion [14]. Producers specify API content only for about 25% of herbal remedies in their registration documents. Usually, the available information is limited to the name of the plant while information on possible impurities and other constituents is missed. Labels of herbs sold in pharmacies, as a rule, do not contain any information regarding the

chemical composition of the medicinal products.

At the same time, the chemical composition of plants of different origins obviously varies depending on the conditions of cultivation (soil, water and harvesting period), as well as plant variety and other factors. A consumer does not know whether the herbs of different producers have a stable composition in the API since this does not follow from the information provided.

The goal of the work was to study the content of API and elemental compositions of samples of SJW medicinal herbs by four different producers, as well as to find any correlations between the content of API and the indicators of the concentrations of chemical elements in plants of various origins.

### Materials and methods

**Instrumentation.** High-performance liquid chromatography (HPLC) according to the State Pharmacopeia of Ukraine (SPU) [19, pp. 86–88] was applied to identify and quantify organic compounds. The research was carried out using a Shimadzu LC-20 liquid chromatograph equipped with a UV detector. Identification of the studied components was performed by absorption spectra and time of substance retention [20]. The following experimental conditions were used: column – Phenomenex Luna C18, 250 mm × 4.6 mm, 5 µm; eluent A – 0.1 % solution of trifluoroacetic acid in high purity water; eluent B – 0.1% solution of trifluoroacetic acid in acetonitrile P; column temperature – 35 °C; flow rate of the mobile phase – 1 ml/min; the volume of the test sample was 5 µl; chromatographic mode - gradient; time of chromatography – 85 min; the wavelength of detection was 368 nm.

Based on the requirements of the SPU [19, pp. 131–132], the suitability of the chromatographic system was checked according to the following indicators: the efficiency of the chromatographic system by the number of theoretical plates, the peak asymmetry coefficient and the mean-square deviation.

Identification and quantitative determination of total hypericins expressed as hypericin was carried out by the spectrophotometry method [21]. The absorbance of the test solution at a wavelength of 590 nm was measured concerning the compensation solution (methanol) using a Hewlett Packard 8452A spectrophotometer. For calculations, the specific absorption rate of hypericin is taken to be 870.

The concentrations of K, Mg, Na, Cu, Zn, Fe, Mn, Pb, Cd, Co, Ni and Cr were measured by the method of flame atomic absorption spectroscopy (FAAS)

using a double-beam Solaar S4 AA Spectrometer (Thermo Electron Co., USA), applying standard conditions in air/acetylene flame and using D<sub>2</sub> correction. The quantification was carried out by external calibration with the use of certified reference materials for metallic ions produced by Bogatsky Physico-Chemical Institute (Odesa, Ukraine). The working range for each element was within a linear range of the method. Calibration intervals were adjusted according to the expected concentrations of elements. The sensitivity of the method concerning each metal was evaluated using the resulted slope of the calibration curves. Measurement of each sample was repeated three times, and the mean value was calculated. Other experimental details were described elsewhere [22; 23].

The content of compounds and chemical elements is given in µg/g. In some graphs, the measured concentrations are reduced by 5, 10 or 100 times (see the corresponding inscriptions in charts) to improve the visibility when comparing the contents of different compounds/elements.

**Sample preparation.** The specimens of herbal remedy, *Hyperici Herba*, commonly known as St John's wort and supplied by four different producers, were bought at local pharmacies in Kyiv, Ukraine. Producers 1, 2, 3 and 4 supply medicinal plants collected in the central, eastern and western parts of the country which are separated by a distance of 400–500 km from each other. Before analysis, the samples were ground in a high-speed rotor mill to store homogeneous samples with grain diameters of ≤1 mm in polyethylene containers.

All chemicals purchased from commercial sources were of analytical grade. Test solutions for chromatographic and spectrophotometric studies were prepared in accordance with the corresponding monographs of the Ukrainian Pharmacopoeia [19; 21]. In order to carry out quantitative measurements, the standard solutions were prepared on the base of standards of chlorogenic acid (product No 25700 of the company Fluka), caffeic acid (No 6773 Fluka), rutin (No 84082 Fluka), hyperoside (No 83388 Fluka), rosemary acid (No 44699 Fluka) and quercetin (No 83370 Fluka).

Samples for FAAS were prepared in the following way. Accurately weighed plant samples (approx. 2 g) were placed into a Teflon reaction crucible and treated with 10 ml of 30 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) / concentrated 65 % HNO<sub>3</sub> (1:4, v/v). Decomposition of the samples was performed in a closed-vessel microwave digestion system. The digestion program consisted of three

stages and was as follows: 80% power for 15 min, 100% for 5 min and 80% power for 20 min. After cooling, the clear digested solutions were transferred quantitatively into clean volumetric flasks and made up to 50 ml with twice distilled water. Blank experiments were carried out in the same way. Three independent digestions were performed for each plant specimen.

**Statistical analysis.** Experimental results were analysed by statistical methods using IBM SPSS Statistics 20 software. All data were tested for normal distribution with the Shapiro–Wilks model and variance homogeneity with the Levene's test. The results were expressed as the means with standard errors of the mean for data with a normal distribution.

Any correlations between organic compounds and element contents were examined with the use of Pearson's correlation coefficients.

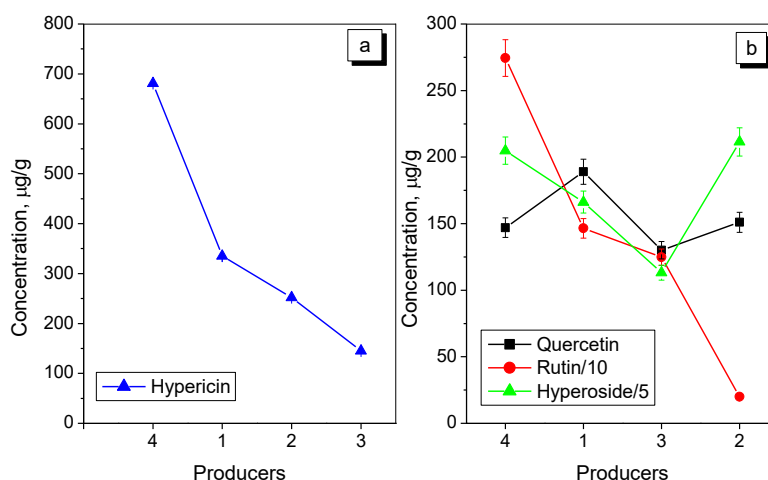
One-way analysis of variance (ANOVA) was used to analyse possible differences among the mean concentrations. The significance level  $p$  was set at or below 5% ( $p \leq 0.05$ ). If the significant differences were found to exist among the means, then post hoc pairwise multiple comparisons were applied to make direct comparisons between two means from two individual groups and determine which means differ. Depending on the results of Levene's tests, either the least significant difference or Tamhane's T2 methods were used in post hoc comparisons for equal or unequal variances respectively.

## Results

Hypericin is among the main APIs in Saint John's wort herbs; its concentration is

shown for samples of different producers in Fig. 1a. The names of producers are arranged along the OX axis to provide a monotonous decrease in the concentration curve. The herb of producer 4 contains four times more hypericin than the herb of producer 3 and more than twice than the samples of producers 1 and 2. However, even the highest measured concentration of hypericin is lower than that regulated in the SPU (0.08%) [21]. Thus, the content of API is not stable; it varies in a wide range and may not consist with the existing requirements for the quality of medicinal plants.

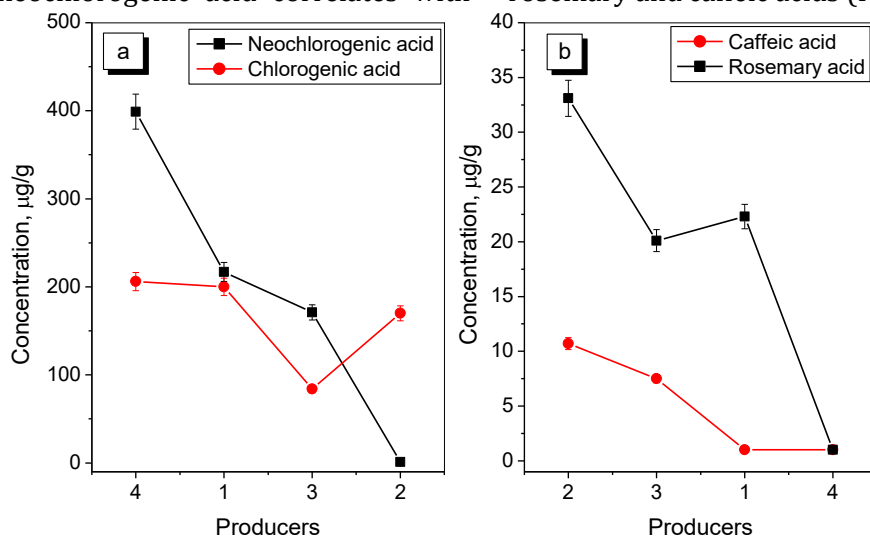
Flavonoids are also essential components of SJW herbs; concentrations of some flavonoids are shown in Fig. 1b. The names of producers are aligned in the same way as in the case of hypericin. Rutin exhibits the most significant variability. Its concentration ranges from 2700  $\mu\text{g/g}$  to 200  $\mu\text{g/g}$ ; the concentration range exceeds an order of magnitude. In contrast to this compound, the concentrations of quercetin and hyperoside appear to be much more stable. Thus, quercetin varies between 130 and 190  $\mu\text{g/g}$ , and hyperoside is between 600  $\mu\text{g/g}$  and 1100  $\mu\text{g/g}$ . According to the SPU specifications, the content of hyperoside should be at least 1.2% [21]. Similar to hypericin, the measured content of one of the major flavonoids in SJW was much lower than the standard value regulated by the SPU. The herb of producer 4 shows the highest levels of both hypericin and rutin. As to the content of hyperoside, the plants of producers 4 and 2 are closest to the standards.



**Fig. 1. Concentrations of hypericin (a) and flavonoids (quercetin, rutin and hyperoside) (b) in SJW herbs of different producers. For the sake of clarity, the rutin and hyperoside concentrations are reduced by factors of 10 and 5, respectively, as inscribed in the legend of graph b**

The contents of four hydroxycinnamic acids studied in the given work are shown in Fig. 2. The content of neochlorogenic acid correlates with

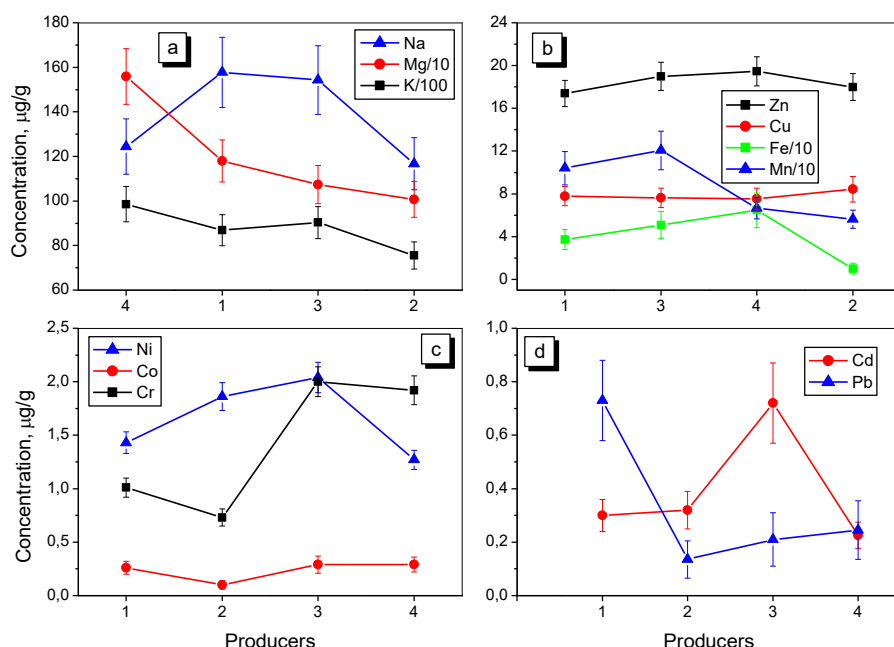
that of chlorogenic acid (Fig. 2a), whereas some signs of correlation are also observed between rosemary and caffeic acids (Fig. 2b).



**Fig. 2.** Concentrations of chlorogenic and neochlorogenic acids (a), as well as rosemary and caffeic acids (b), in SJW herbs of different producers

The herb of producer 4 is characterised by both the highest concentrations of chlorogenic and neochlorogenic acids and the actual absence of rosemary and caffeic acids. On the contrary, the herbs of producers 2 and 3 demonstrate maximal levels of caffeic and rosemary acids, while the contents of hypericin, flavonoids and chlorogenic acids are minimal.

In addition to bioactive organic compounds, the content of some essential macro- and microelements, as well as toxic metals, is determined in the herb samples. The results are shown separately for essential macroelements (Fig. 3a), main microelements (Fig. 3b) and some essential trace elements (Fig. 3c), as well as toxic metals (Fig. 3d).



**Fig. 3.** Elemental composition of SJW herb samples of different producers: a – K, Na and Mg; b – Zn, Cu, Mn and Fe; c – Ni, Co, and Cr; and d – Pb and Cd. For the sake of clarity, the concentrations of K and concentrations of Mg, Fe and Mn are reduced by factors of 100 and 10, respectively, as inscribed in the legends of graphs a and b

The concentration of K in different herbs remains relatively stable: The difference between maximum and minimum levels does not exceed 30%. In contrast, the Na and Mg concentrations

can vary in a broader range (Fig. 3a), the maximum-to-minimum ratio is equal to 1.5. Also, stability is demonstrated by Zn and Cu (Fig. 3b), which was already found in previous studies [22].

An anticorrelation between the concentrations of Fe and Mn is observed in the samples of producers 1, 3 and 4, but is violated in the case of sample 2.

Concentrations of other essential microelements (Co, Ni and Cr) range from 0.2% to 2% (Fig. 3c) that is in agreement with the literature data [24–26]. The content of toxic metals is relatively stable for samples 2, 3 and 4 as regards lead and 1, 2 and 4 concerning cadmium (Fig. 3d). The concentrations of Pb and Cd increase in samples of producers 1 and 3, respectively.

A detailed comparison of the experimental data obtained with the literature data, carried out in work [22], in general, indicates the consistency of these results, although the concentration range observed in the literature is much broader than measured in this paper.

## Discussion

The fact that the content of bioactive substances in samples of different origins varies widely is among the essential experimental findings. Also, the measured concentrations do not entirely comply with the requirements of the SPU. The diversity in the chemical composition among wild populations of plants can depend on geographic origins and environmental conditions [27, 28], population and genetics [28, 29]. Also, the variation is affected by the floral development stages and harvesting season [30–32]. The proper storage of collected herbs is also of importance because the concentrations of bioactive compounds are sensitive to light, pH and temperature [33]. Observed concentration ranges together with pharmacopoeia standards and some literature data are shown for some bioactive compounds in Table 1.

**Table 1**  
**Concentrations of bioactive substances: measured in this work, available from the literature and standardised by Pharmacopoeias of the EU and Ukraine**

Name	Concentration range from the literature data, µg/g	Measured concentrations, µg/g	Pharmacopoeia of EU, % (µg/g)	SPU, % (µg/g)
Quercetin	300-1300 [32]	130-190		
Hyperoside	5000-20000 [34]	560-1060		≥1.2% (≥12000)
Rutin	3000-16000 [34]	200-2750	Extract: ≥6% (60000)	
Hypericin	500-3000 [34]	140-680	Extract: 0.1-0.3% (1000-3000)	≥0.08% (≥800)

Pharmaceutical production is based on the construction of a pharmaceutical quality system. Such a system is single and agreed set of measures designed to ensure the quality of pharmaceutical products unconditionally at all stages of their production, as well as at subsequent stages of their storage and distribution. In Ukraine, the circulation of medicinal plants is regulated by a series of guidelines that are recommendatory and are listed in [14]. However, a single quality system has not yet been developed and implemented. Standardisation issues are also unresolved. A similar situation is observed in other countries of the world [35; 36]. The experimental data presented here illustrate the possible consequences of such a state of affairs when the quality of medicinal plants is far from being stable.

At the same time, many people consider medicinal herbs to be safer than synthetic medicines despite a lack of good scientific evidence for such a conclusion. For example, 85% of respondents take one or more natural health products, including medicinal herbs and herbal medicines, and 47% consider them as safer than prescription drugs [37]. In reality, herbal

medicines are always chemically complex, and challenges remain to neutralise differences between products made of the same medicinal herbs and ensure safety and similar therapeutic effects.

Another finding relates to the interaction of bioactive compounds with metal ions. The Pearson's correlation coefficients were calculated, and the related significance values *p* were determined for all pairs of 11 metals and eight bioactive compounds (Table 2). In total, eight interacting pairs were found to demonstrate statistically significant, either positive or negative correlations. In four other cases, the values *p* < 0.1, varying from 0.065 to 0.08, that is, the estimated significances are at the level of statistical tendency.

In addition to the metal ion – API interactions, some statistically significant correlations were recently observed between various organic compounds, in particular between rutin and hydroxycinnamic acids [38].

Four of the five metals, which are part of these correlation pairs, are involved in the biochemical processes in plants and are usually referred to as



essential macro- (K and Mg) and microelements (Fe and Mn). Cadmium belongs to a group of toxic impurities which are not necessary for the development of plants and often fall into them from the outside in various ways [22]. As shown in Fig. 3, the concentrations of toxic impurities and microelements in SJW samples can vary in a wide range. Iron shows a positive correlation with

rutin; therefore, the increased content of Fe can contribute to increased rutin concentration in medicinal herbs. In two other cases, the relationships are negative. Thus, the increase in Mn concentration from 60 µg/g to 120 µg/g (Fig. 3b) is accompanied by a drop in the content of hyperoside from 1050 µg/g to 580 µg/g (Fig. 1b).

Table 2

**The Pearson's correlation coefficients are calculated for correlations between concentrations of bioactive substances and chemical elements in SJW herb samples of different producers. The relevant significances (2-tailed) are shown in parentheses**

	Chlorogenic acid	Rutin	Hyperoside	Quercetin	Neochlorogenic acid	Caffeic acid	Rosemary acid	Hypericin
Mg	0.558 (0.442)	0.947* (0.050)	0.350 (0.65)	0.033 (0.967)	0.935 (0.065)	-0.771 (0.229)	-0.951* (0.049)	0.954* (0.046)
Zn	-0.263 (0.737)	0.600 (0.402)	-0.085 (0.915)	-0.786 (0.214)	0.563 (0.437)	-0.084 (0.916)	-0.735 (0.265)	0.437 (0.563)
Cu	0.076 (0.924)	-0.871 (0.13)	0.46 (0.54)	0.119 (0.881)	-0.880 (0.120)	0.730 (0.270)	0.840 (0.160)	-0.419 (0.581)
Na	-0.372 (0.628)	0.094 (0.906)	-0.841 (0.159)	0.288 (0.712)	0.139 (0.861)	-0.350 (0.650)	0.032 (0.968)	-0.425 (0.575)
K	0.089 (0.911)	0.961* (0.039)	-0.211 (0.79)	-0.174 (0.826)	0.959* (0.041)	-0.743 (0.257)	-0.960* (0.040)	0.640 (0.360)
Ni	-0.885 (0.115)	-0.741 (0.259)	-0.53 (0.47)	-0.569 (0.431)	-0.750 (0.250)	0.870 (0.130)	0.659 (0.341)	-0.888 (0.112)
Fe	-0.03 (0.97)	0.921 (0.079)	-0.315 (0.685)	-0.246 (0.754)	0.920 (0.080)	-0.683 (0.317)	-0.926 (0.074)	0.547 (0.453)
Co	-0.133 (0.867)	0.803 (0.197)	-0.559 (0.441)	-0.059 (0.941)	0.820 (0.180)	-0.719 (0.281)	-0.754 (0.246)	0.307 (0.693)
Mn	-0.608 (0.392)	0.06 (0.94)	-0.960* (0.04)	0.003 (0.997)	0.095 (0.905)	-0.179 (0.821)	0.019 (0.981)	-0.517 (0.483)
Cd	-0.993** (0.007)	-0.469 (0.531)	-0.768 (0.232)	-0.65 (0.35)	-0.474 (0.526)	0.654 (0.346)	0.395 (0.605)	-0.812 (0.188)
Pb	0.368 (0.632)	0.094 (0.906)	-0.187 (0.813)	0.894 (0.106)	0.145 (0.855)	-0.598 (0.402)	0.097 (0.903)	-0.051 (0.949)

\* All statistically significant correlations are marked with asterisks (\* for  $p < 0.05$  and \*\* for  $p < 0.01$ )

Similarly, the concentration of chlorogenic acid is influenced by Cd: the twofold increase in the concentration of this metal (Fig. 3d) leads to a 2.4 drop of the level of chlorogenic acid (Fig. 2b).

By the norms of the World Health Organisation (WHO), the maximum values for heavy metals in medicinal plants oral intake are as follows: less than 10 µg/g and 0.3 µg/g for Pb and Cd, respectively [39]. The measured concentrations of Pb do not exceed the WHO norm (Fig. 3d). At the same time, Cd levels exceed permissible limits given by the WHO in several investigated samples (Fig. 3d). Such a result agrees with other literature data confirming the fact that *Hypericum* species are hyperaccumulators of Cd [25, 40]. Therefore, determination of toxic metals, especially Cd, in *Hypericum* species used as raw material for herbal medicines and dietary supplements is of high priority.

The revealed correlations between the concentrations of metallic ions and some bioactive

compounds give evidence to possible interactions between them. According to [40], the interaction of bioactive compounds and metal ions can be reduced to three main processes.

Some studies showed links between the content of secondary metabolites in SJW and the elements in the growth medium. For example, Ni exposure was found to suppress the production of hypericin and pseudohypericin [41]. In contrast, Cr treatment of the nutrient medium increases the concentrations of some hypericins in seedlings of SJW [42]. The most significant impact was observed at a concentration of 0.1 mM Cr(VI): pseudohypericin rose by +379% during seven days of such a treatment and protopseudohypericin increased by +404%. No changes were detected in the content of hypericin.

Also, an interaction between elements and bioactive compounds can alter their bioactivity, for example, by the formation of metal-organic complexes with flavonoids and hypericin [43; 44].



Besides, the complexing of metal ions with organic compounds controls their bioavailability [7; 9; 45].

Such effects could be used to enhance the yield of bioactive compounds and thus to make herbal medicines more potent, allowing for less product being contained in a dosage. For example [9], complexes of flavonoids and metal ions usually act as antioxidants, which are often more potent than the parent molecules. However, they may be pro-oxidants as well. Such complexes may act as chemoprotective agents, but sometimes they enhance the oxidative damage of biomolecules. Such examples illustrate a complicated interdependence between the ligand, the metal ion and the surrounding environment when interactions between all these objects result in specific properties of a given complex.

Medicinal plants usually represent complex systems. Under conditions of the absence of reference standards, instability in the chemical composition can start from the stage of collection of crude plants and further deepen during processing and storage. Therapeutic results and safety issues can vary significantly from product to product, even within a single class of herbal medication. All benefits and risks revealed in a particular herbal remedy cannot be easily extrapolated to other similar products, as is the case for synthetic pharmaceutical substances. Therefore, it is difficult to establish quality control parameters and maintain consistent quality for each batch of herbal medicines.

Nevertheless, all active pharmaceutical ingredients are chemicals, whether synthesised in plants or chemical laboratories, therefore, all they, irrespective of their source, should be controlled with the same accuracy and adjusted by similar standards of quality regarding identity, purity, and stability. Reliable and consistent quality is the basis of efficacy and safety of herbal medicinal products. For specific therapeutic purposes, it may be useful to use purified extracts with one or two of the major components. In any case, a more thorough analysis of medicinal plants and standardisation of the conditions of cultivation, collection and drying of crude plants to ensure the content of API of a specific concentration are still topical challenges that lie before the modern pharmacognosy.

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### Conclusions

The content of bioactive substances, including total hypericins, flavonoids, and hydroxycinnamic acids, as well as essential micro and macro elements and toxic metals, was studied in Saint John's wort herbs of four different producers of Ukraine by the methods of high-performance liquid chromatography and flame atomic absorption spectroscopy.

The content of total hypericins varies by a factor of 5 from a sample to sample. The Investigated flavonoids also show the variability of their concentrations: The difference between the maximum and minimum levels of rutin reaches a factor of 10 and hyperoside – 2. The concentration of quercetin varies from sample to sample in a narrower range compared to other bioactive substances: its fluctuations do not exceed 30%.

The concentrations of hypericin and hyperoside, regulated by the State Pharmacopeia of Ukraine, appeared to be lower than the standard values.

Elemental composition is also characterised by variability. Changes in the concentration of essential macroelements (K, Mg and Na) and some of the microelements (Zn, Cu and Ni) do not exceed 30-40%. The concentration of other essential microelements (Fe, Mn, Co and Cr) varies between samples of different origin in much broader limits - from a factor of 2 (Mn) to 6 (Fe).

Some samples are enriched with both Pb and Cd to the level of 0.7 µg/g, while, in most cases, the Pb and Cd contents are in the ranges of 0.15-0.25 µg/g and 0.25-0.35 µg/g, respectively. The concentration of Pb does not exceed the WHO norm (10 µg/g) in all samples, while the Cd content is higher than the WHO norm (0.3 µg/g) in three out of four studied samples.

The presence of statistically significant correlations between the individual elements (K, Mg, Fe, Mn and Cd) and organic compounds (hypericin, rutin, hyperoside, and neochlorogenic, chlorogenic and rosemary acids) was found by the method of Pearson's pair correlations. From a practical viewpoint, the most important are positive correlations of Fe - rutin, as well as negative correlations between Mn and hyperoside and Cd with chlorogenic acid.

The experimental results obtained indicate that the existing requirements and norms for medicinal plants in Ukraine, which are sold in the pharmacy network, do not guarantee the stability

of their chemical composition and, accordingly, the stability of their therapeutic properties. Therefore, it is advisable to introduce a more thorough analysis of medicinal plants, standardise the conditions of cultivation, collection and drying of crude plants to ensure the content of API of a particular concentration, as well as a more detailed description on the packaging of the composition and effect of herbs on the human body.

## Bibliography

- [1] Barnes J. St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties / J. Barnes, L. A. Anderson, J. D. Phillipson // *Journal of Pharmacy and Pharmacology*. – 2001. – Vol. 53, N5. – P. 583–600.
- [2] Bridi H. Structural diversity and biological activities of phloroglucinol derivatives from *Hypericum* species / H. Bridi, G. de Carvalho Meirelles, G. Lino von Poser // *Phytochemistry*. – 2018. – Vol. 155. – P. 203–232.
- [3] Butterweck V. St. John's wort: Role of active compounds for its mechanism of action and efficacy / V. Butterweck, M. Schmidt // *Wien Med Wochenschrift*. – 2007. – Vol. 157, N13–14. – P. 356–361.
- [4] Wölfl U. Topical Application of St. John's Wort (*Hypericum perforatum*) / U. Wölfl, G. Seellinger, C. M. Schempp // *Planta Medica*. – 2014. – Vol. 80, N2. – P. 109–120.
- [5] A systematic review of St. John's wort for major depressive disorder / E. A. Apaydin, A. R. Maher, R. Shanman [et. al.]. // *Systematic Reviews*. – 2016. – Vol. 5. – P. 148–173.
- [6] De C. Brito L. An overview of anticancer activity of *Garcinia* and *Hypericum* / L. de C. Brito, A. L. Rangel Berenger, M. R. Figueiredo // *Food and Chemical Toxicology*. – 2017. – Vol. 109, N2. – P. 847–862.
- [7] Symonowicz M. Flavonoids and their properties to form chelate complexes / M. Symonowicz, M. Kolanek // *Biotechnology and Food Sciences*. – 2012. – Vol. 76, N1. – P. 35–41.
- [8] Flavonoids as Antioxidants and Developmental Regulators: Relative Significance in Plants and Humans / C. Brunetti, M. Di Ferdinando, A. Fini [et. al.]. // *International Journal of Molecular Sciences*. – 2013. – Vol. 14, N2. – P. 3540–3555.
- [9] Kasprzak M. M. Properties and applications of flavonoid metal complexes / M. M. Kasprzak, A. Erxleben, J. Ochocki // *RSC Advances*. – 2015. – Vol. 5. – P. 45853–45877.
- [10] Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals / I. B. Afanas'eva, E. A. Ostrakhovitch, E. V. Mikhal'chik [et. al.]. // *Biochemical Pharmacology*. – 2001. – Vol. 61, N6. – P. 677–684.
- [11] Kuntic V. Effects of Rutin and Hesperidin and their Al(III) and Cu(II) Complexes on in Vitro Plasma Coagulation Assays / V. Kuntic, I. Filipovic, Z. Vujic // *Molecules*. – 2011. – Vol. 16, N2. – P. 1378–1388.
- [12] Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice / A.-S. Cho, S.-M. Jeon, M.-J. Kim [et. al.]. // *Food and Chemical Toxicology*. – 2010. – Vol. 48, N3. – P. 937–943.
- [13] Rajendra Prasad N. Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line / N. Rajendra Prasad, A. Karthikeyan, S. Karthikeyan, B. V. Reddy // *Molecular and Cellular Biochemistry*. – 2011. – Vol. 349, N1–2. – P. 11–19.
- [14] Баула О. П. Забезпечення якості лікарських засобів рослинного походження: стан та перспективи / О. П. Баула, Т. М. Деркач // *Фармацевтичний часопис*. – 2017. – № 2. – С. 79–86.
- [15] St John's wort (*Hypericum perforatum*) products – an assessment of their authenticity and quality / A. Booker, A. Agapouda, D. A. Frommenwiler [et. al.]. // *Phytomedicine*. – 2018. – Vol. 40. – P. 158–164.
- [16] Choudhary N. An overview of advances in the standardization of herbal drugs / N. Choudhary, B. Singh Sekhon // *Journal of Pharmaceutical Education and Research*. – 2011. – Vol. 2, N2. – P. 55–70.
- [17] Kaushik D. Current issues in Authentication and Quality control of Natural Products / D. Kaushik, M. K. Pandey, A. Sharma // *Research in Plant Biology*. – 2014. – Vol. 4, N5. – P. 57–64.
- [18] Derkach, T. Pharmacopoeia Methods for Elemental Analysis of Medicines: a Comparative Study / T. Derkach, O. Baula // *Bulletin of Dnipropetrovsk University. Series Chemistry*. – 2017. – Vol. 25, N2. – P. 73–83.
- [19] Державна Фармакопея України: в 3 т. / ДП «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид., 1 том. – Харків: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2015. – 1128 с.
- [20] Гудзенко А. В. Дослідження речовин-маркерів для розробки методик контролю якості лікарських рослин, їх сумішей та полікомпонентних фітопрепаратів [дисертація]. Київ: ДУ «Інститут фармакології та токсикології НАМН України»; 2014. – 421 с.
- [21] Державна Фармакопея України: в 3 т. / ДП «Український науковий фармакопейний центр якості лікарських засобів». – 2-е вид., 3 том. – Харків: ДП «Український науковий фармакопейний центр якості лікарських засобів», 2014. – 732 с.
- [22] Derkach T.M. Essential and Toxic Microelements in the Medicinal Remedy *Hyperichi herba* by Different Producers / T. M. Derkach, V. G. Khomenko // *Research Journal of Pharmacy and Technology*. – 2018. – Vol. 11, N2. – P. 466–474.
- [23] Derkach T. Elemental Composition of the Medicinal Plants *Hypericum perforatum*, *Urtica dioica* and *Matricaria chamomilla* Grown in Ukraine: A Comparative Study / T. Derkach, V. Khomenko // *Pharmacognosy Journal*. – 2018. – Vol. 10, N3. – P. 486–491.
- [24] Elgin Cebe G. Calcium and Magnesium Contents in Three *Hypericum* L. Species from Turkey / G. Elgin Cebe, Ö. Söğüt // *Journal of Medicinal Food*. – 2018. – Vol. 21, N8. – P. 819–822.
- [25] Elemental fingerprinting of *Hypericum perforatum* (St John's Wort) herb and preparations using ICP-OES and chemometrics / J. Owen, S. B. Kirton, S. J. Evans, J. Stair // *Journal of Pharmaceutical and Biomedical Analysis*. – 2016. – Vol. 125. – P. 15–21.
- [26] The Assessment of Toxic Metals in Plants Used in Cosmetics and Cosmetology / A. Fischer, B. Brodziak-Dopierała, K. Loska, J. Stojko // *International Journal of Environmental Research and Public Health*. – 2017. – Vol. 14. – P. 1280–1292.
- [27] Changes in the content of bioactive substances among *Hypericum montbretii* populations from Turkey /

- C. Cirak, J. Radusiene, L. Ivanauskas [et. al.]. // Revista Brasileira de Farmacognosia. – 2014. – Vol. 24, N1. – P. 20–24.
- [28] The impact of media composition on production of flavonoids in agitated shoot cultures of the three *Hypericum perforatum* L. cultivars 'Elixir,' 'Helos,' and 'Topas' / I. Kwiecien, J. Smolin, L. Beerhues, H. Ekiert // In Vitro Cellular & Developmental Biology – Plant. – 2018. – Vol. 54. – P. 332–340.
- [29] Comparison of chemical composition of *Hypericum perforatum* and *H. maculatum* in Estonia / L. Rusaalepp, A. Raal, T. Püssa, U. Mäeorg // Biochemical Systematics and Ecology. – 2017. – Vol. 73. – P. 41–46.
- [30] Antioxidant capacity, bioactive compounds, and volatile constituents of flowers of *Hypericum perforatum* at different harvest stages / L. Ge, Y.-Y. Yao, T.-L. Kang [et. al.]. // Acta Prataculturae Sinica. – 2017. – Vol. 26, N9. – P. 66–74.
- [31] Evaluating the content of active principles from wild *hypericum perforatum* l. in various harvesting seasons/ E. Ionescu, T. V. Iordache, C. E. Tebrencu [et. al.]. // Revista de Chimie. – 2018. – Vol. 69, N7. – P. 1892–1897.
- [32] Variation in concentrations of major bioactive compounds in *Hypericum perforatum* L. from Lithuania / E. Bagdonaitė, P. Mártonfi, M. Repčák, J. Labokas // Industrial Crops and Products. – 2012. – Vol. 35, N1. – P. 302–308.
- [33] The quantitative effects of temperature and light intensity on hyperforin and hypericins accumulation in *Hypericum perforatum* L. / M. S. Odabas, J. Radusiene, N. Camas [et. al.]. // Journal of Medicinal Plants Research. – 2009. – Vol. 3, N7. – P. 519–525.
- [34] St. John's Wort (*Hypericum* Spp.) – Relation between the biological source and medical properties / N. Kladar, B. Srđenović, N. Grujić [et. al.]. // *Hypericum: Botanical Sources, Medical Properties and Health Effects*; H. R. Davis (ed.). – Nova Science Publishers, Inc.: N.-Y., USA, 2015. – P. 53–80.
- [35] Booker A. Value Chains of Botanicals and Herbal Medicinal Products: A European Perspective / A. Booker, M. Heinrich // Herbal Gram. – 2016. – Vol. 112. – P. 40–45.
- [36] Ghosh D. Quality issues of herbal medicines: internal and external factors / D. Ghosh // International Journal of Complementary & Alternative Medicine. – 2018. – Vol. 11, N1. – P. 67–69.
- [37] Barry A.R. Patients' perceptions and use of natural health products / A. R. Barry // Canadian Pharmacists Journal. – 2018. – Vol. 151, N4. – P. 254–262.
- [38] Вміст біологічно активних речовин та елементний склад трави звіробою різних виробників / Т. М. Деркач, В. В. Страшний, О. О. Старікова, С. М. Лисенко // Фармацевтичний часопис. – 2018. – № 4. – С. 17–25.
- [39] WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO Press: Geneva, Switzerland, 2007. – 105 p.
- [40] Owen J. D. Investigation of the Elemental Profiles of *Hypericum perforatum* as used in herbal remedies / J. D. Owen // PhD thesis. University of Hertfordshire, UK, March 2013, 205 p. Available from: <https://core.ac.uk/download/pdf/29840202.pdf>
- [41] Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum*) / S. J. Murch, K. Haq, H. P. V. Rupasinghe, P. K. Saxena // Environmental and Experimental Botany. – 2002. – Vol. 49. – P. 251–257.
- [42] Induction of hypericins in *Hypericum perforatum* in response to chromium / B. Tirillini, A. Ricci, G. Pintore [et. al.]. // Fitoterapia. – 2006. – Vol. 77, N3. – P. 164–70.
- [43] de Souza R. F. V. Antioxidant properties of complexes of flavonoids with metal ions / R. F. V. De Souza, W. F. De Giovanni // Redox Report. – 2004. – Vol. 9, N2. – P. 97–104.
- [44] Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application / D. Xu, M. J. Hu, Y. Q. Wang, Y. L. Cui // Molecules. – 2019. – Vol. 24, N6. – P. 1123–1138.
- [45] Bravo A. Metal complexes of the flavonoid quercetin: antibacterial properties / A. Bravo, J. R. Anaconda // Transition Metal Chemistry. – 2001. – Vol. 26, N1. – P. 20–23.

## References

- [1] Barnes, J., Anderson, L. A., Phillipson, J. D. (2001). St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. J Pharm Pharmacol, 53(5), 583–600.  
<https://doi.org/10.1211/0022357011775910>
- [2] Bridi, H., de Carvalho Meirelles, G., Lino von Poser, G. (2018). Structural diversity and biological activities of phloroglucinol derivatives from *Hypericum* species. Phytochem, 155, 203–232.  
<https://doi.org/10.1016/j.phytochem.2018.08.002>
- [3] Butterweck, V., Schmidt, M. (2007). St. John's wort: Role of active compounds for its mechanism of action and efficacy. Wien Med Wochenschr, 157(13–14), 356–361.  
<https://doi.org/10.1007/s10354-007-0440-8>
- [4] Wölflle, U., Seellinger, G., Schempp, C.M. (2014). Topical Application of St. John's Wort (*Hypericum perforatum*). Planta Med, 80(2), 109–120.  
<http://dx.doi.org/10.1055/s-0033-1351019>
- [5] Apaydin, E. A., Maher, A. R., Shanman, R., Booth, M. S., Miles, J. N. V., Sorbero, M. E., Hempel, S. (2016). A systematic review of St. John's wort for major depressive disorder. Syst Rev, 5, 148–173.  
<https://doi.org/10.1186/s13643-016-0325-2>
- [6] De C. Brito, L., Rangel Berenger, A.L., Figueiredo, M. R. (2017). An overview of anticancer activity of *Garcinia* and *Hypericum*. Food Chem Toxicol, 109(2), 847–862.  
<https://doi.org/10.1016/j.fct.2017.03.053>
- [7] Symonowicz, M., Kolanek, M. (2012). Flavonoids and their properties to form chelate complexes. Biotechnol Food Sci, 76(1), 35–41.
- [8] Brunetti, C., Di Ferdinando, M., Fini, A., Pollastri, S., Tattini, M. (2013). Flavonoids as Antioxidants and Developmental Regulators: Relative Significance in Plants and Humans. Int J Mol Sci, 14(2), 3540–3555.  
<https://doi.org/10.3390/ijms14023540>
- [9] Kasprzak, M. M., Erxleben, A., Ochocki, J. (2015). Properties and applications of flavonoid metal complexes. RSC Adv, 5, 45853–45877.  
<https://doi.org/10.1039/C5RA05069C>
- [10] Afanas'eva, I. B., Ostrakhovitch, E. A., Mikhail'chik, E. V., Ibragimova, G. A., Korkina, L.G. (2001). Enhancement of antioxidant and anti-inflammatory activities of bioflavonoid rutin by complexation with transition metals. Biochem Pharmacol, 61(6), 677–684.
- [11] Kuntic, V., Filipovic, I., Vujic, Z. (2011). Effects of Rutin and Hesperidin and their Al(III) and Cu(II) Complexes on in Vitro Plasma Coagulation Assays. Molecules, 16(2), 1378–1388.  
<https://doi.org/10.3390/molecules16021378>

- [12] Cho, A.-S., Jeon, S.-M., Kim, M.-J., Yeo, J., Seo, K.-I., Choi, M.-S., Lee, M.-K. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food Chem. Toxicol.*, 48(3), 937–943. <https://doi.org/10.1016/j.fct.2010.01.003>
- [13] Rajendra Prasad, N., Karthikeyan, A., Karthikeyan, S., Reddy, B.V. (2011). Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. *Mol Cell Biochem*, 349(1–2), 11–19. <https://doi.org/10.1007/s11010-010-0655-7>.
- [14] Baula, O.P., Derkach, T.M. (2017). [Quality assurance of herbal medicinal products: status and prospects]. *Farmatsevtichnyi chasopys – Pharmaceutical Review*, 2, 79–86 (in Ukrainian). <https://doi.org/10.11603/2312-0967.2017.2.7816>
- [15] Booker, A., Agapouda, A., Frommenwiler, D.A., Scotti, F., Reich, E., Heinrich, M. (2018). St John's wort (*Hypericum perforatum*) products – an assessment of their authenticity and quality. *Phytomedicine*, 40, 158–164. <https://doi.org/10.1016/j.phymed.2017.12.012>.
- [16] Choudhary, N., Singh Sekhon, B. (2011). An overview of advances in the standardization of herbal drugs. *J. Pharm Educ. Res.*, 2(2), 55–70.
- [17] Kaushik, D., Pandey, M.K., Sharma, A. (2014). Current issues in Authentication and Quality control of Natural Products. *Res. Plant. Biol.*, 4(5), 57–64.
- [18] Derkach, T., Baula, O. (2017) *Pharmacopoeia Methods for Elemental Analysis of Medicines: a Comparative Study*. Bull Dnipropetrovsk Univ. Ser. Chem., 25(2), 73–83. <https://doi.org/10.15421/081711>.
- [19] Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. [State Pharmacopoeia of Ukraine]. 2<sup>nd</sup> ed., Vol. 1. Kharkiv: Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines; 2015. (in Ukrainian).
- [20] Hudzenko, A.V. [Investigation of marker substances for the development of methods for quality control of medicinal plants, their mixtures and polycomponent phytopreparations]. Kyiv: DU «Institut farmakologicheskoy toksykologicheskoy NAMN Ukrainy», 2014. (in Ukrainian).
- [21] Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines. [State Pharmacopoeia of Ukraine]. 2<sup>nd</sup> ed., Vol. 3. Kharkiv: Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines; 2014. (in Ukrainian).
- [22] Derkach, T.M., Khomenko, V.G. (2018). Essential and Toxic Microelements in the Medicinal Remedy *Hyperichi herba* by Different Producers. *Res. J. Pharm. Technol.*, 11(2), 466–474. <https://doi.org/10.5958/0974-360X.2018.00086.0>
- [23] Derkach, T., Khomenko, V. (2018). Elemental Composition of the Medicinal Plants *Hypericum perforatum*, *Urtica dioica* and *Matricaria chamomilla* Grown in Ukraine: A Comparative Study. *Pharmacogn J.*, 10(3), 486–491. <https://doi.org/10.5530/pj.2018.3.80>
- [24] Elgin Cebe, G., Söğüt, Ö. (2018). Calcium and Magnesium Contents in Three *Hypericum L.* Species from Turkey. *J. Med. Food*, 21(8), 819–822. <https://doi.org/10.1089/jmf.2017.0117>
- [25] Owen, J., Kirton, S.B., Evans, S.J., Stair, J. (2016). Elemental fingerprinting of *Hypericum perforatum* (St John's Wort) herb and preparations using ICP-OES and chemometrics. *J. Pharm. Biomed. Anal.*, 125, 15–21. <https://doi.org/10.1016/j.jpba.2016.02.054>
- [26] Fischer, A., Brodziak-Dopierała, B., Loska, K., Stojko, J. (2017). The Assessment of Toxic Metals in Plants Used in Cosmetics and Cosmetology. *Int J Environ Res Public Health*, 14, 1280–1292. <https://doi.org/10.3390/ijerph14101280>
- [27] Cirak, C., Radusiene, J., Ivanauskas, L., Jakstas, V., Čamas, N. (2014). Changes in the content of bioactive substances among *Hypericum montbretii* populations from Turkey. *Rev Bras Farmacogn*, 24(1), 20–24. <http://dx.doi.org/10.1590/0102-695X20142413352>
- [28] Kwiecień, I., Smolin, J., Beerhues, L., Ekiert, H. (2018). The impact of media composition on production of flavonoids in agitated shoot cultures of the three *Hypericum perforatum L.* cultivars 'Elixir,' 'Helos,' and 'Topas'. *In Vitro Cell Dev Biol Plant*, 54, 332–340. <https://doi.org/10.1007/s11627-018-9900-7>
- [29] Rusalepp, L., Raal, A., Püssa, T., Mäeorg, U. (2017). Comparison of chemical composition of *Hypericum perforatum* and *H. maculatum* in Estonia. *Biochem. Syst. Ecol.*, 73, 41–46. <https://doi.org/10.1016/j.bse.2017.06.004>
- [30] Ge, L., Yao, Y.-Y., Kang, T.-L., Li, J.-Y., He, H.-J., Yang, D.-L., Li, M.-F. (2017). Antioxidant capacity, bioactive compounds, and volatile constituents of flowers of *Hypericum perforatum* at different harvest stages. *Acta Prataculturae Sinica*, 26(9), 66–74. <https://doi.org/10.11686/cyxb2016484>
- [31] Ionescu, E., Iordache, T.V., Tebrencu, C.E., Cretu, R.M., Florea, A.M., Radu, A.L., Zaharia, A., Sarbu, A. (2018). Evaluating the content of active principles from wild *hypericum perforatum l.* in various harvesting seasons. *Revista de Chimie*, 69(7), 1892–1897.
- [32] Bagdonaitė, E., Mártonfi, P., Repčák, M., Labokas, J. (2012). Variation in concentrations of major bioactive compounds in *Hypericum perforatum L.* from Lithuania. *Ind. Crops. Prod.*, 35(1), 302–308. <https://doi.org/10.1016/j.indcrop.2011.07.018>.
- [33] Odabas, M.S., Radusiene, J., Čamas, N., Janulis, V., Ivanauskas, L., Cirak, C. (2009). The quantitative effects of temperature and light intensity on hyperforin and hypericins accumulation in *Hypericum perforatum L.* *J. Med. Plant. Res.*, 3(7), 519–525.
- [34] Kladar, N., Srđenović, B., Grujić, N., Rat, M., Gavarić, N., Anačkov, G., Božin, B. (2015). St. John's Wort (*Hypericum Spp.*) – Relation between the biological source and medical properties. In: H. R. Davis (Ed.), *Hypericum: Botanical Sources, Medical Properties and Health Effects* (pp. 53–80) New York, USA: Nova Science Publishers, Inc.
- [35] Booker, A., Heinrich, M. (2016). Value Chains of Botanicals and Herbal Medicinal Products: A European Perspective. *Herbal Gram.*, 112, 40–45.
- [36] Ghosh, D. (2018). Quality issues of herbal medicines: internal and external factors. *Int J Complement Alt Med*, 11(1), 67–69. <https://doi.org/10.15406/ijcam.2018.11.00350>
- [37] Barry, A.R. (2018). Patients' perceptions and use of natural health products. *Can. Pharm. J. (Ott)*, 151(4), 254–262. <https://doi.org/10.1177/1715163518779409>
- [38] Derkach, T.M., Strashnyi, V.V., Starikova, O.O., Lysenko, S.M. (2018). [Content of bioactive substances and elemental composition of Saint John's wort herbs of different producers]. *Farmatsevtichnyi chasopys – Pharmaceutical Review*, 4, 17–25 (in Ukrainian). <https://doi.org/10.11603/2312-0967.2018.4.9576>
- [39] WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO Press: Geneva, Switzerland, 2007.
- [40] Owen, J.D. (2013). Investigation of the Elemental Profiles of *Hypericum perforatum* as used in herbal remedies. PhD thesis. University of Hertfordshire, UK. Available from: <https://core.ac.uk/download/pdf/29840202.pdf>

- 
- [41] Murch, S. J., Haq, K., Rupasinghe, H. P. V., Saxena, P. K. (2002). Nickel contamination affects growth and secondary metabolite composition of St. John's wort (*Hypericum perforatum*). *Environ Exp Botany*, 49, 251-257. [http://dx.doi.org/10.1016/S0098-8472\(02\)00090-4](http://dx.doi.org/10.1016/S0098-8472(02)00090-4)
- [42] Tirillini, B., Ricci, A., Pintore, G., Chessa, M., Sighinolfi, S. (2006). Induction of hypericins in *Hypericum perforatum* in response to chromium. *Fitoterapia*, 77(3), 164-70. <https://doi.org/10.1016/j.fitote.2006.01.011>
- [43] de Souza, R. F. V., De Giovani, W. F. (2004). Antioxidant properties of complexes of flavonoids with metal ions. *Redox Report*, 9(2), 97-104. <https://doi.org/10.1179/135100004225003897>
- [44] Xu, D., Hu, M. J., Wang, Y. Q., Cui, Y. L. (2019). Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. *Molecules*, 24(6), 1123-1138. <https://doi.org/10.3390/molecules24061123>
- [45] Bravo, A., Anaconda, J. R. (2001). Metal complexes of the flavonoid quercetin: antibacterial properties. *Transit. Met. Chem.*, 26(1), 20-23. <https://doi.org/10.1023/A:1007128325639>