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THE MICRONUTRIENT PROFILE OF MEDICINAL PLANT EXTRACTS

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ABSTRACT
Medicinal plants contain biologically active substances that have a physiological effect on the human body. In the territory of Ukraine, 15 of the most important medicinal plants grow from a medical point of view, among which are *Melissa officinalis* L and *Calendula officinalis*. Micronutrients are necessary for the body in small quantities, not being a source of energy, they take part in their assimilation, as well as in the regulation of various functions and the implementation of the processes of growth and development of the human body. The study aims to establish the micronutrient profile of extracts and infusions from medicinal raw materials – *Calendula officinalis* and *Melissa officinalis*. The established micronutrient profile includes data on the content of such macro- and microelements as calcium, potassium, sodium, iron, zinc, and copper in extracts and infusions from *Melissa officinalis* L officinalis L and *Calendula officinalis*. Sodium predominates from certain macronutrients, the superiority of which is manifested in *Calendula officinalis* when infused. A large amount of calcium also passes into the aqueous-alcoholic infusion from *Calendula officinalis*. Copper and zinc prevail among the determined microelements in water extracts of *Calendula officinalis*. Comparing the results obtained, we can say in the affirmative about the micro- and macro elements that have passed into extracts that *Calendula officinalis* is richer in these substances. Because infusions and extracts are recommended to be added as an additional ingredient to vegetable and fruit juices, their positive infusion on the human body will increase the recommended daily requirement of potassium and sodium. Based on the results of this study, extracts and infusions of *Melissa officinalis* L and *Calendula officinalis* can be considered as an essential source of micronutrients for enriching fruit and vegetable juices in canned food for health purposes.

Keywords: micronutrients; biologically active substances; extract; *Calendula officinalis; Melissa officinalis* L

INTRODUCTION
The body is not able to synthesize trace elements and makes up for their deficiency only from food, therefore trace elements are irreplaceable, and their absence or excess causes the development of many diseases **(Ebid, 2015)**. For thousands of years, people have received mineral elements only with food in an organically bound form. The entire system of digestion, active transport, and assimilation of our body is focused precisely on the consumption of organically bound macro- and microelements. This system not only controls the assimilation process, depending on the needs of the body at a given moment but also has a mechanism for the accumulation of vital organically "connected elements in special" cellular depots **(Liaqat et al., 2016)**.

Unfortunately, our system of consumption of elements is not at all adapted to the assimilation of inorganic compounds and salts of various elements that enter our body with water, food, air as a product of technogenic pollution **(Mushtruk et al., 2020a)**. For the same reason, due to the inadequacy of our assimilation system and the absence of special mechanisms for assimilation, transport, and accumulation, in most cases inorganic forms of macro- and microelement compounds have an extremely low toxicity threshold and, at the slightest excess of the dosage, cause severe intoxication of all vital organs and systems. That is why, for example, preparations of inorganic iodine and selenium are prescribed only under medical supervision **(Park and Kim, 2016)**.

Preparations containing inorganic microelements are prescription pharmaceuticals and are taken only under medical supervision. Extracts and infusions of biologically active substances from medicinal raw materials, compensate for the deficiency of macro- and microelements, contain, as a rule, only organically bound elements, are not able to harm the body even with thoughtless and incorrect use **(Waheed et al., 2018)**.

The peculiarity of extracts and infusions from medicinal plants is that their biologically active substances are in a certain ratio, contributing to the optimal effect on the human body. Some components of plant extracts are similar in chemical structure to physiologically active substances of the body (hormones, vitamins, enzymes, etc.). Therefore, such natural medicines are more actively involved in the biochemical processes of the human body together with juices **(Sheiko et al., 2019)**.

The need to fortify food with micro- and microelements is dictated by objective changes in the lifestyle, set, and nutritional value of the food used in food **(Mushtruk et al., 2020b)**. To provide the human body with the necessary micronutrients, food should be varied, and products are rich in biologically active substances, therefore, the addition of extracts and infusions to any juice will allow the use of beverages in food that significantly restore human health and reduce various diseases.

Scientific hypothesis

Is to determine some micro- and macroelements in medicinal plants, data for which are not available in the literature, to create tables of the chemical composition of medicinal plants. There is an assumption that *Calendula officinalis* is richer in substances that were determined by tests, due to their intense coloration. Studies of the number of micronutrients of extracts and infusions from *Melissa officinalis* L and *Calendula officinalis* should be carried out using flame photometry and spectrometry methods for their exact amounts. Surely the transition of micronutrients into infusions and extracts will also be different due to the different

MATERIAL AND METHODOLOGY

For research, we took medicinal raw materials – *Calendula officinalis* and *Melissa officinalis L*. Calendula flowers and melissa leaves were used for research. According to the manufacturer, the plants were collected at the initial stage of flowering. Dried and ready for research raw materials were purchased in a pharmacy network. For experimental studies, the raw material was ground on a laboratory crusher. Then added aqueous and aqueous-alcoholic solutions at hydromodule 1, ie the same amount of raw material and extractant. Extraction of micronutrients or their infusion was carried out according to the methods presented in the research results.

Chemicals

Distilled water (H_2O) for extraction, it was pre-purified in an aqueous distiller. The electrical conductivity of manufactured distilled water meets the national standard of laboratory distilled water of the first level and can be used in medicine and everyday life.

Ethanol (C_2H_5OH, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

Sodium chloride ($NaCl$, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

Potassium chloride (KCl, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

Calcium carbonate ($CaCO_3$, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

The above anhydrous salts were used to make reference solutions and to construct calibration graphs of macronutrient content.

Animals and Biological Material

Medicinal raw materials (*Melissa officinalis* L and *Calendula officinalis*) for research (provided by Natalia farm, Khmelnytsky region, Shepetivka district).

Instruments

Automated refractometer (Anton Paar, producer «Inter-Synthesis» Limited Liability Company, Ukraine). By which the dry matter content was determined.

The thermometer is laboratory (TLS-200, producer «Inter-Synthesis» Limited Liability Company, Ukraine).

Surfactometer (Saturn-4, producer «Inter-Synthesis» Limited Liability Company, Ukraine). By which the content of trace elements was determined.

Flame photometer (BWB Technologies, producer «Inter-Synthesis» Limited Liability Company, Ukraine).

Laboratory Methods

Determination of dry matter content was carried out according to **ISO 2173:2003 (2003)**. The method of flame photometry is based on the construction of a calibration graph. It is used when the composition of the sample and the approximate amount of interfering ions are known. This makes it possible to bring the composition of the reference solutions as close as possible to the composition of the determined samples. Quantitative determination of elements by flame, photometry is possible only in the presence of reference solutions.

For the determination of macroelements, the flame photometry method was used, which is an arbitration method for the determination of metals in food products.

Emission photometry is based on the method of constructing a calibration graph. It is used when the composition of the sample is known, and the approximate amount of ion interferes. This makes it possible to maximally approximate the composition of the standard solutions to the composition of the samples to be determined. Standard solutions were prepared from standard solutions by dilution. Water was used as an extract, and a 20% aqueous-alcoholic solution was used for infusion.

Reference solutions are solutions (samples) in which the content of the element to be determined is known with sufficient accuracy. Reference solutions were prepared from standard solutions by diluting them. The starting materials for the preparation of reference solutions are double-distilled water and anhydrous chemically pure salts of the brand "HC" or $NaCl$, KCl, and $CaCO_3$. The calculated amount of salt was weighed on an analytical balance to the nearest 0.0001 g and dissolved in a volumetric flask. For each solution (both reference and determined) took 5 readings of the device and found the average value.

Calibration graphs were constructed in the coordinates:

$$I = f(C) \tag{1}$$

where: I is the current on the microammeter (instrument readings), µA;
C is the concentration of the solution, $\mu g.mL^{-1}$.

The number of the element in the sample was determined according to the schedule. Determination of trace elements using the atomic absorption method was carried out according to **GOST 30178 (1996)** (Table 1).

Description of the Experiment

Sample preparation: Two samples of medicinal raw materials were used for research – *Melissa officinalis* L and

Calendula officinalis, which were purchased at the pharmacy.

Number of samples analyzed: During the experimental research, 20 different samples (*Melissa officinalis* L and *Calendula officinalis*) of one manufacturer purchased in different pharmacy chains were examined.

Number of repeated analyses: All measurements of instrument, readings were performed 5 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was also 5 times.

Statistical Analysis

Mathematical and statistical processing of experimental data was carried out in determining the criteria of Cochran's C test, Fisher, and Student's *t*-test. The accuracy of the data was determined using the Cochrane criterion, and the adequacy of the mathematical model was checked using the Fisher and Student criteria. Statistical processing was performed in Microsoft Excel 2016 values were estimated using mean and standard deviations and subsequently evaluated in the statistical program XL Stat. In hypothesis testing, if the *p*-value is lower than significant level, in case of XL Stat software by Addinsoft (version 2019.3.2), it is 0.05, the null hypothesis was rejected and alternativehypothesis was confirmed.

RESULTS AND DISCUSSION

The established micronutrient profile includes data on the content of such macro- and microelements as calcium, potassium, sodium, iron, zinc, and copper in extracts and infusions from *Melissa officinalis* L and *Calendula officinalis*. Sodium predominates from certain macronutrients, the superiority of which is manifested in *Calendula officinalis* when infused. A large amount of calcium also passes into the aqueous-alcoholic infusion from *Calendula officinalis*. Copper and zinc prevail among the determined microelements in water extracts of *Calendula officinalis*. The following scientists were engaged in similar researches in works **(Spiegler, Liebau and Hensel, 2017; Teanpaisan et al., 2017)** investigated the process of extraction of *Matricaria recutita*, and in works **(Tiwary et al., 2017; Veni, Pushpanathan and Mohanraj, 2017**) similar process of *Plantago lanceolata* was investigated.

Comparing the results obtained, we can say in the affirmative about the micro- and macro elements that have passed into extracts that *Calendula officinalis* is richer in these substances. Because infusions and extracts are recommended to be added as an additional ingredient to vegetable and fruit juices, their positive infusion on the human body will increase the recommended daily requirement for potassium and sodium by 4 – 5%, calcium by 2 – 3%, iron by 15 – 17%, zinc, and copper up to 20% with a portion of the drink in the amount of 0.00025 m^3.

Before starting the experiments, the raw material was preliminarily crushed for better transfer of the raw material substances into the extract or infusion. When using water, the crushed raw material was subjected to an increase in temperature from 20 – 70 ºC, determining the dry matter content every 10 minutes (Figure 1). In scientific works **(Bazarnova et al., 2017; Bezerra et al., 2018)**, temperature regimes above 70 ºC were used, and the dry matter content was recognized after the completion of the process, which in our opinion can lead to mixing of dry matter content. After 40 minutes from the beginning of the experiments, the dry matter content did not increase, which indicates swollen raw materials. In this case, the hydronic modulus was 1. Therefore, it was decided to carry out the extraction process by maintaining the mixture for some time and at a certain temperature. These two factors will be the main ones in the process of the conducted research.

For the transfer of extractives from medicinal raw materials to the extract, the temperature range was taken from 40 – 60 ºC. The authors of scientific works **(Konovalenko and Polovko, 2019; Florea et al., 2020)** took the temperature range from 30 – 40 ºC, applying the settling process for 24 hours, which in our opinion can lead to mixing of dry matter content.

As seen in Figure 2 at a temperature of 60 ºC and keeping such an extract at 60 minutes, the value of the dry matter content is of the greatest importance, and the continuation of experiments does not make sense. The authors of scientific works **(Makasana et al., 2017; Ranjith, 2018; Mamadalieva et al., 2019; D'Auria, Mecca and Todaro, 2020)** performed similar experiments at a temperature of 75 ºC and for 2 hours but the maximum dry matter yield is in the range of 6.5 – 7.2%.

For another extractant of a water-alcohol solution, thanks to which fat-soluble substances can be extracted, a concentration of 20 wt % was taken. When using a water-alcohol solution, the temperature of the mixture was not increased, as it was done for water, since alcohol is a volatile substance. Measurements were carried out every 30 minutes and the dry matter content was determined over 2.5 hours. Based on the research results, curves were constructed for both medicinal plants (Figure 3). The authors of the following scientific papers **(Ulusoy, Acıdereli and Tutar, 2017; Chronopoulou et al., 2019)** investigated the extraction of fat-soluble substances at different concentrations of water-alcohol solution using the following ratios of water: alcohol 1: 3, 1: 6 and 1: 9. **Melfi et al. (2018)** and **Rathi et al. (2017)** investigated the extraction of fat-soluble substances using liquid and gas, such a process and its technological aspects will be covered in future research.

It is visible on the graph the transition of dry substances into infusions, and for *Calendula officinalis*, it took 2 hours for the dry matter content to increase to 9%, and for *Melissa officinalis* L – 1.5 hours, but the maximum amount of dry matter was 8.8%. Therefore, the best time regimen for infusion in a water-alcohol solution with a concentration of 20% wt. at hydro module 1 and a process temperature of 60 ºC for *Melissa officinalis* L it is 1.5 hours, and for *Calendula officinalis* – 2 hours. But the authors of scientific works **(Gullón et al., 2017; Al-Snafi, 2020; Jadhav et al., 2020; Zhang et al., 2020)** argue that the best time regimen for infusion in a water-alcohol solution with a concentration in the range of 5 – 15% wt. with a hydro module of 0.2 – 0.75 and a process temperature of 45 – 55 ºC for *Melissa officinalis* L and *Calendula officinalis* is 1 – 3 hours but the maximum dry matter yield is in the range of 4.5 – 6.2%.

Table 1 Conditions for atomic absorption determination of elements.

Element	Resonance line length, nm	Spectral slit width, nm	Concentration of solutions for graduation, μg.mL^{-1}
Cu (II) (copper)	324.7	0.1	0.5 – 4.0
Zn (II) (zinc)	213.9	0.5	0.1 – 2.0
Fe (III) (iron)	248.3	0.1	1 – 10

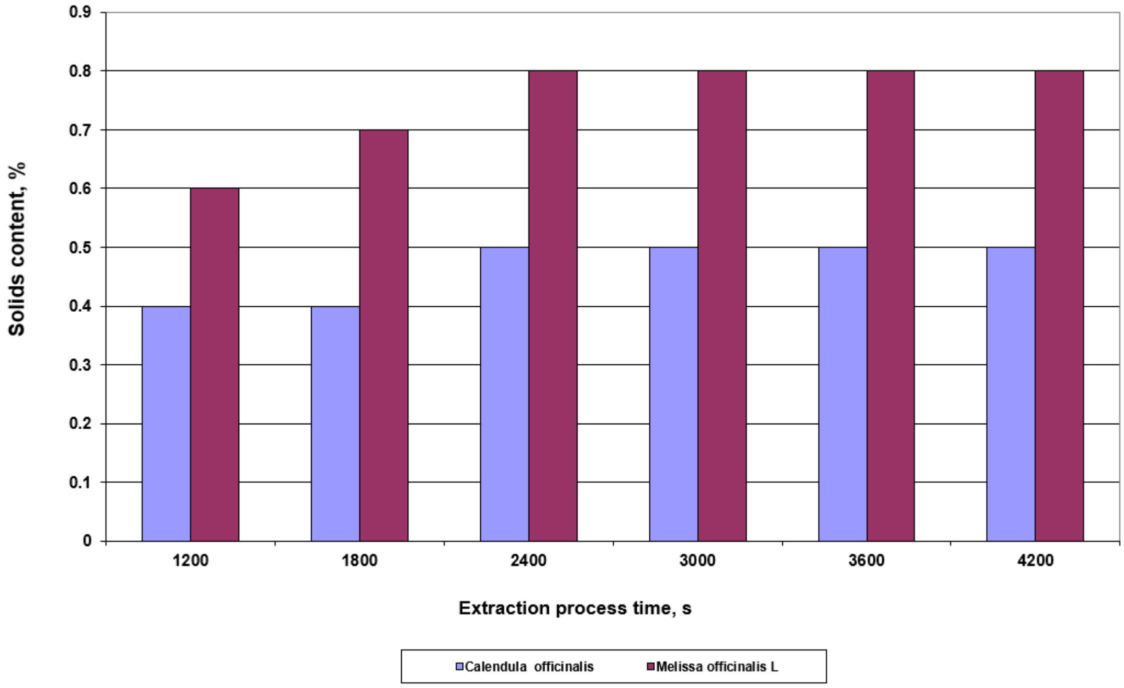

Figure 1 Change in the number of dry substances in medicinal raw materials with an increase in the duration of extraction.

Table 2 The content of macronutrients in extrtacts and infusions, μg.mL^{-1}.

Element	Water extract		Water-alcohol infusion	
	Calendula officinalis	***Melissa officinalis L***	***Calendula officinalis***	***Melissa officinalis L***
K (potassium)	1.5 ±0.01	1.27 ±0.01	1.2 ±0.01	1.05 ±0.01
Ca (calcium)	20 ±0.1	16 ±0.05	36 ±0.15	23.5 ±0.1
Na (sodium)	280 ±5	148 ±3	275 ±3	12 ±1

Table 3 Content of trace elements in extracts and infusions, μg.mL^{-1}.

Trace element	Water extract		Water-alcohol infusion	
	Calendula officinalis	***Melissa officinalis L***	***Calendula officinalis***	***Melissa officinalis L***
Zn (zinc)	1.03 ±0.01	0.3 ±0.01	0.48 ±0.01	0.23 ±0.01
Cu (copper)	0.73 ±0.01	0.18 ±0.01	0.6 ±0.01	0.2 ±0.01
Fe(III) (iron)	3.8 ±0.015	0.32 ±0.015	0.2±0.01	0.03 ±0.015

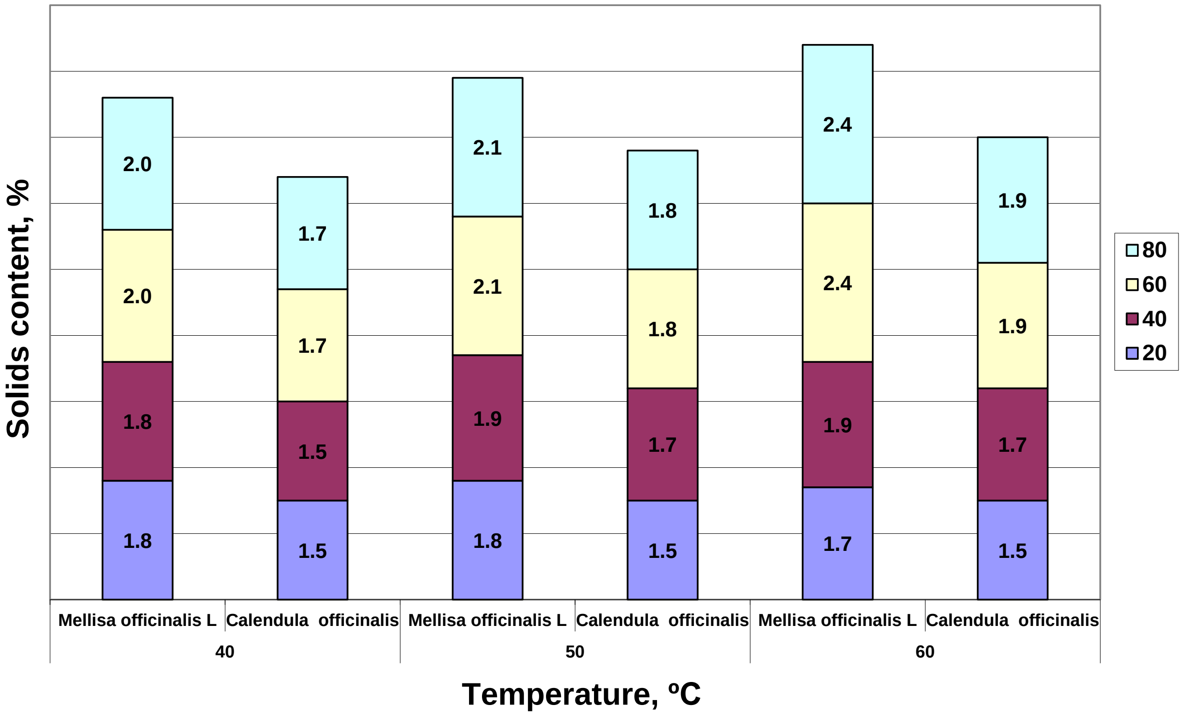

Figure 2 Change in the content of dry substances in medicinal raw materials with an increase in the duration of time at different temperatures.

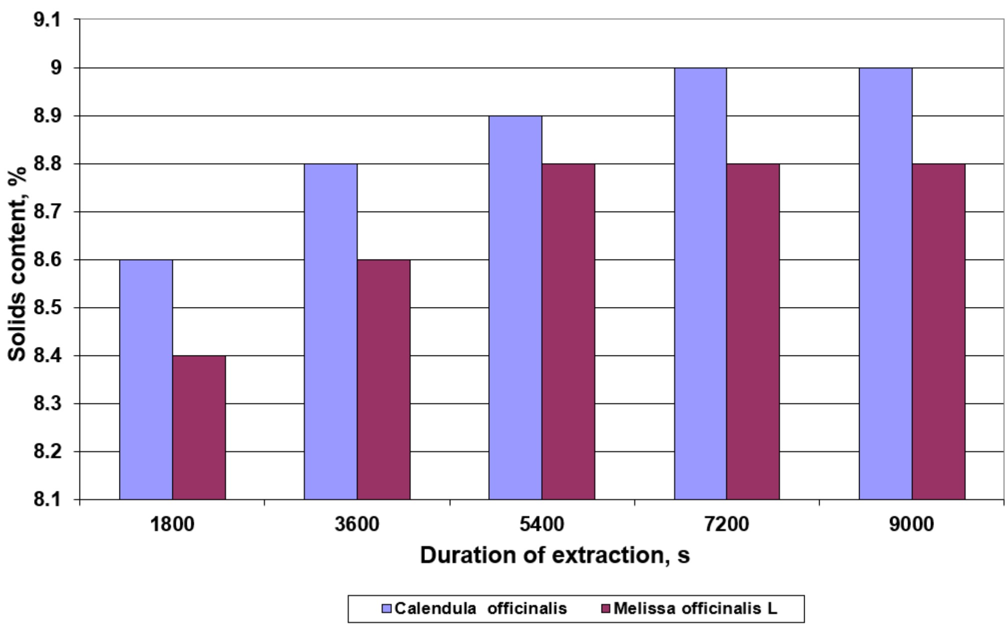

Figure 3 Influence of the duration of infusion of medicinal raw materials in a water-alcohol solution on the dry matter content.

As shown by the results of our research (Table 2), at optimal parameters of extraction and infusion, the content of such macroelements as potassium, calcium, and sodium in aqueous and aqueous-alcoholic solutions slightly fluctuates, but the order remains the same. Except for the amount of sodium in *Melissa officinalis* L, more than 12 times more of this element passes into the aqueous extract, while calcium, on the contrary, is less for both *Melissa officinalis* L and *Calendula officinalis*.

Data analysis Table 3 indicates a higher content of the transferred zinc, copper, and iron in the aqueous extract than their amount in the aqueous-alcoholic solution. Especially noteworthy is a large amount of iron, which, after extraction, passes into an aqueous solution (19 times more). Considering that the necessary dose of all these substances is greater for the normal life of the human body, we can confidently talk about the benefits of using such extracts from *Melissa officinalis* L and *Calendula officinalis*, which will have a beneficial effect on the development and growth of the human body.

These data are consistent with the results of studies of the composition of macro- and microelements for medicinal raw materials and, in particular, lie within the data obtained at different extraction temperatures **(Gupta et al., 2018;**

Bober et al., 2020). Among the micronutrients, the amounts of ascorbic acid were also determined, which passed into extracts and infusions from *Calendula officinalis* and *Melissa officinalis L*. Based on the results obtained, it can be argued that the maximum amount of this substance passes into the aqueous extract of *Melissa officinalis*, which corresponds to 6.2 mg.100g^{-1} after 1 hour of extraction. An increase in the amount of ascorbic acid in an aqueous-alcoholic infusion of *Melissa officinalis* L up to 4.2 mg.100g^{-1} is also less important for 1 hour of infusion, but this amount is more than for the extract and infusion from *Calendula officinalis* by 56 and 55%, respectively. The extraction time of ascorbic acid from *Calendula officinalis* was 1 hour, and the infusion time was 2 hours.

The composition of micronutrients in extracts and infusions from medicinal raw materials corresponds to the standards of consumption for adults recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization **(Zheplinska et al., 2020)**. The information provided in the micronutrient profile can be used for non-commercial communications and cannot be used for other purposes, including product labeling. The data obtained can be useful for creating tables of the chemical composition of extracts and infusions of such medicinal raw materials as *Calendula officinalis* and *Melissa officinalis L*. There is a need for further scientific research on this topic to determine other biologically active substances and generalize the results on the amount of these substances in extracts and infusions from medicinal raw materials. The relevance and novelty of the research are confirmed by obtaining patents of Ukraine for a useful model for vegetable drinks containing extracts and infusions from these types of medicinal raw materials **(Palamarchuk et al., 2019; Zheplinska et al., 2019)**. Because infusions and extracts are recommended to be added as an additional ingredient to vegetable and fruit juices, their positive infusion on the human body will increase the recommended daily requirement of potassium and sodium by 4 – 5%, calcium by 2 – 3%, iron by 15 – 17%, zinc, and copper up to 20% with a portion of the drink in the amount of 0.00025 m^3.

CONCLUSION

The amounts of micronutrients of extracts and infusions from *Melissa officinalis* L and *Calendula officinalis* were investigated using flame photometry and spectrometry methods. The established micronutrient profile includes data on the content of such macro- and microelements as calcium, potassium, sodium, iron, zinc, and copper in extracts and infusions from *Melissa officinalis* L and *Calendula officinalis*. Sodium predominates from certain macronutrients, the superiority of which is manifested in *Calendula officinalis* when infused. A large amount of calcium also passes into the aqueous-alcoholic infusion from *Calendula officinalis*. Copper and zinc prevail among the determined microelements in water extracts of *Calendula officinalis*. Comparing the results obtained, we can say in the affirmative about the micro- and macro elements that have passed into extracts that *Calendula officinalis* is richer in these substances.

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