CHEMISTRY AND TECHNOLOGY OF MEDICINAL COMPOUNDS AND BIOLOGICALLY ACTIVE SUBSTANCES

ХИМИЯ И ТЕХНОЛОГИЯ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И БИОЛОГИЧЕСКИ АКТИВНЫХ СОЕДИНЕНИЙ

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RESEARCH ARTICLE

Method of electrochemical biotesting for comparative analysis of probiotic and antibiotic properties of various plant extracts

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Objectives. *The purpose of this study was to develop an objective instrumental method for assessing microbial contamination and expressing the probiotic and antibiotic properties of food, pharmacological, and other products.*

Methods. *The developed method consists of periodic (every 2 h) registration of changes in pH, redox potential, and electrical conductivity of a liquid nutrient medium incubated in the presence and absence of viable test microorganisms and test samples.*

Results. *Using liquefied CO_2 from 10 different types of plant materials, we carried out a comparative analysis of probiotic and antibiotic activities against Lactobacillus acidophilus of various concentrations of subcritical whole extracts obtained.*

Conclusions. *Among the studied plant extracts, the most active prolonged antibiotic properties were exhibited by extracts from the leaves of Eucalyptus globulus Labill. and seeds of Illicium verum Hook.f. at a concentration in the test medium (C_{TE}) more than 3 vol %, whereas the most active prolonged probiotic properties were exhibited by an extract from the herb of Mentha arvensis L. at C_{TE} = 0.2 vol %. In most cases, the initial antibiotic activity of the tested extracts (TEs) was greater than their prolonged activity. Also, the mid-term (in terms of TEs interaction time with test microorganisms) antibiotic activity of TEs was intermediate in value between their initial and prolonged activity. In the test medium, the decreasing concentration of TEs decreases their antibiotic activity monotonically and increases their probiotic activity, suggesting that the biological activity of products, including various plant extracts, is largely determined not only by the raw material and the method of extracting biologically active substances from it but also by the concentration of the extract in the product and by the interaction time of the said product with microbiota and others. In most cases, a significant number of tests could establish the exact nature*

of these dependencies. The proposed method is much more rapid, objective, and informative and less laborious and material-intensive than using standard microbiological methods in assessing the initial microbial contamination and the probiotic and antibiotic properties of various samples of both the new and already approved pharmaceuticals, foods, and other products, as well as the individual ingredients and additives.

Keywords: *microbiological biotesting, antibiotic properties, plant extracts, microbiological contamination, electrochemical methods*

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НАУЧНАЯ СТАТЬЯ

Методика электрохимического биотестирования для сравнительного анализа про- и антибиотических свойств различных экстрактов

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Цели. *Разработать быструю и объективную инструментальную методику оценки микробной обсемененности, а также про- и антибиотических свойств различных образцов пищевой, фармакологической и иной продукции.*
Методы. *Разработанная методика заключается в периодической (через каждые 2 ч) регистрации изменений pH, редокс потенциала и электропроводности жидкой питательной среды, инкубируемой в присутствии и в отсутствие жизнеспособных тестовых микроорганизмов и тестируемых образцов.*
Результаты. *С помощью представленной методики проведён сравнительный анализ про- и антибиотической активности в отношении Lactobacillus acidophilus разных концентраций цельных докритических экстрактов, полученных с помощью сжиженного CO_2 из 10 различных видов растительного сырья.*
Выводы. *Проведенные исследования показали, что среди исследованных растительных экстрактов наиболее активные пролонгированные антибиотические свойства проявили экстракты из листьев эвкалипта шаровидного (Eucalyptus globulus Labill.) и семян бадьяна настоящего (Illicium verum Hook.f.) при их концентрации в тестовой среде ($C_{ТЭ}$) больше 3 об.%; а наиболее активные пролонгированные пробиотические свойства проявил экстракт из травы мяты луговой (Mentha arvensis L.) при $C_{ТЭ}$ = 0.2 об.%. Начальная антибиотическая активность тестированных экстрактов (ТЭ) в большинстве случаев была больше их пролонгированной активности. В то время как среднесрочная (по времени взаимодействия ТЭ с тестовыми микроорганизмами) антибиотическая активность ТЭ как правило была промежуточной по величине между их начальной и пролонгированной активностью. При этом с уменьшением концентраций ТЭ в тестовой среде их антибиотическая активность монотонно уменьшалась, а пробиотическая активность увеличивалась. Таким образом очевидно, что биологическая активность продукции, включающей различные растительные экстракты, в значительной степени определяется не только сырьём и способом экстрагирования из него биологически активных веществ, но и концентрацией экстракта в продукции, а также временем взаимодействия упомянутой*

продукции с микробиотой и т.п. Причем точный характер этих зависимостей в большинстве случаев может быть установлен лишь с помощью значительного числа тестовых испытаний. Последние удобно проводить с помощью представленной в этой работе методики, которая позволяет существенно более быстро, объективно и информативно, а также существенно менее трудоёмко и материалоёмко, чем при использовании стандартных микробиологических методов, оценивать исходную микробную обсемененность, а также про- и антибиотические свойства различных образцов как новой, так и уже допущенной к употреблению продукции, а также отдельных ингредиентов и добавок к оной.

Ключевые слова: *биотестирование микробиологическое, антибиотические свойства, экстракты растительные, микробная обсемененность, электрохимические методы*

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INTRODUCTION

The development of objective, fast, and available for a wide range of applications methods for the quantitative assessment of the probiotic and antibiotic properties of a large number of samples of new and approved-to-use products in pharmaceutical, cosmetic, food, feed, and other sectors of the national economy is becoming increasingly important. The abovementioned methods are one of the important components of the product quality and safety monitoring system. In the implementation of the abovementioned methods, both multicellular and unicellular test living organisms are used. Unicellular living organisms are used both because of they are the cheapest, available and statistically reliable model of living organisms, in general, and because they are also the model of useful natural human microbiota and natural microbiota that can cause various infectious diseases, toxicosis, allergic reactions, and contribute toward the spoilage of food and other products.

However, more time, materials, and labor of qualified personnel are required in standard microbiological testing (visual assessment of the overall survival of microorganisms or the size of the zone of growth retardation of their colonies), resulting in incomplete, subjective, and static information on disorders of the vital functions of test organisms [1–3]. Thus, the use of instrumental technologies in microbiological testing seems promising. Among them, optical and electrochemical methods are simple in execution, reliable, and universal.

In products manufactured and consumed by human society, there is an increasing lack of biologically active substances (BAS) of natural origin that contribute to the normal development and functioning of the human body (the human body is only weakened by stresses, presence of various physical and chemical factors of environmental pollution, lack of natural lighting and physical activity, and contacts with numerous extraneous microbiota, etc.) and symbiotically associated beneficial microbiota, or suppress microbiota's vital activity that is harmful to humans.

Because of the complexity of achieving the desired degree of purity, stereospecificity, and other parameters, the production of concentrated synthetic analogs of BAS at the current level of technology development to achieve a sufficiently high degree of biological activity of such compounds is costly and ineffective. In addition, BAS of natural origin has significantly lower side effects in terms of the spectrum width and intensity of action to humans and other living organisms compared with synthetic agents. Main sources of BAS that are used as functional additives to pharmaceuticals, foods, and other products are extracts and essential oils obtained from various plant materials.

Essential oils obtained by distillation or cold or hot pressing of plant raw materials [4] give the opportunity to achieve a significantly greater and stable biological activity of the final product compared with aqueous, alcoholic, and other plant extracts (PEs) obtained without removing extractants. Therefore, essential oils are now widely used as (i) additives to foods, pharmaceuticals, cosmetics, and other products, which have selective or low-specific promicrobial or antimicrobial actions, (ii) additives with various types of normalizing action used, inter alia, in the treatment of various diseases, and (iii) preserving, antioxidant, aromatizing, flavoring, and other types of additives [3, 4–13]. In addition,

essential oils are used as antiseptics, environmental-friendly insecticides and pesticides, and additives to various dental, wound-healing, and other medical and packaging materials [5, 14–19].

However, only sufficient volatile and thermostable substances can be extracted from raw materials by distillation, and only a little yield of the final product is achieved by pressing, which is too critical to the raw materials. Therefore, recently the extracts obtained from plant materials via the subsequent removal of the extractant from the final product by increasing temperature and lowering pressure are used instead of using essentials oils. Currently, PEs obtained using liquefied carbon dioxide (CO_2PE) as an extractant are the most common. Liquefied carbon dioxide is then completely removed from the final product by changing the pressure and temperature of the latter [20–26].

Particularly, *Biozevtica* (Dedovsk, Moscow oblast, Russia)[1] not only has developed but also put into production, followed by a fairly widespread sale, a whole line of yogurts, mayonnaise, vegetable and butter oils, spicy mixtures (dry, fat, or water-soluble), juices, lemonades, and other products with additives of various CO_2PEs (which is also produced by *Biozevtica*).

In comparison with essential oils, CO_2PEs have a significantly wider variety of BAS included in their composition. If the extraction is carried out with pressure above 7.6 MPa and a temperature of the carbon dioxide (CO_2) below 31°C, the extracts are called "subcritical." Otherwise, the extracts are called "supercritical" because the CO_2, being in a supercritical state, exhibits both the liquid and gas properties. Besides, CO_2PEs are categorized into "selective" (obtained at low CO_2 pressures and having a composition closer to essential oils) and "whole" (obtained at high CO_2 pressures). In addition to volatile components common for essential oils, whole subcritical CO_2PEs (the richest in various BAS) contain heavier plant resins, paraffins, pigments, and others and have more viscous pasty consistency compared with "ordinary" essential oils; however, they are easily dissolved by both ethers and vegetable oils (although, in some cases, slight heating is required to dissolve them).

The purpose of this study was to develop a quick and objective instrumental method for assessing the microbial contamination and expressing the probiotic and antibiotic properties of various products, as well as individual ingredients and additives, followed by a comparative analysis using the developed method for the influence of various PEs on the dynamics of the human microbiota.

[1] URL: https://biozevtika.ru. Accessed October 06, 2020.

MATERIALS AND METHODS

We took whole subcritical extracts produced by *Kazanskii zavod ekstraktov* (Kazan, Russia)[2] using liquefied CO_2 at a pressure of 7.3 MPa and a temperature of 20°C from the following types of plant materials: leaves of southern blue gum (*Eucalyptus globulus Labill.*) (No. 1), clove buds (cloves, *Syzygium aromaticum L. Merr. & L.M. Perry*) (No. 2), ginger roots (*Zingiber officinale Roscoe*) (No. 3), shoots of common sage (*Salvia officinalis L.*) (No. 4), creeping thyme herb (thyme, *Thymus serpyllum L.*) (No. 5), oregano herb (*Origanum vulgare L.*) (No. 6), field mint herb (*Mentha arvensis L.*) (No. 7), shoots of rosemary (*Rosmarinus officinalis L.*) (No. 8), star anise seeds (star anise, *Illicium verum Hook.f.*) (No. 9), and cardamom green seeds (*Elettaria cardamomum L. Maton*) (No. 10). This plant was chosen because it is currently the largest producer in Russia of CO_2PEs, which are used as sources of dietary supplements in medicine, veterinary medicine, cosmetic and food products, household chemicals, etc. Raw material No. 1 was obtained from Australia; raw materials Nos. 2, 3, and 8–10 were from India, and raw materials Nos. 4 and 5–7 were from Russia (Krasnodar krai and Kazan precincts, respectively).

On the basis of the existing our developments on various methods of instrumental biotesting [27–33], to analyze the effect of different concentrations of the tested extracts (TEs) on the dynamics of the vital activity of microorganisms, we developed the method, as described in the following.

Four series of measurements were carried out for each batch of TE, and before the beginning of each batch, a nutrient medium was prepared, which was a sterile aqueous solution with pH 7.2 ± 0.2, containing 5-g/L glucose, 20-g/L protein hydrolyzate, and 2-g/L NaCl. The nutrient medium was then inoculated with *Lactobacillus acidophilus* ATCC 4356, which were selected as typical representatives of the microbiota because they are widespread both outside and inside the human body and other living organisms, which are actively participating in the destruction of various biopolymers, and also because they are widely used by humans in many biotechnological processes, including bioconservation, ensiling, and obtaining various fermented milk products. Then, the mentioned culture medium with test microorganisms was incubated at 37.0 ± 0.1°C until the content of viable microorganisms reached approximately 5×10^6 cells/mL, which was verified by a nephelometric method using a bacterial turbidity standard.

The obtained test medium was poured into the test measuring capacities (MCs), in which the amount

[2] URL: https://extract.market. Accessed October 06, 2020.

of TE required to achieve its specified concentration in the test medium was previously added each (three MCs in parallel). In this case, we used the following as control (which was also placed in the MC in triplicate): a test medium with viable microorganisms without TE (control-1) and a solution with a given concentration of TEs in a sterile nutrient medium (control-2).

Then both the test and all control MCs were incubated at 37.0 ± 0.1°C for another 6 h. The pH, redox potential (*E*, mV) and specific linear low-frequency electrical conductivity (*X*, mS/sm) values of the test media contained in each of the MCs were sequentially recorded with an interval of 2 h. In this case, the pH and E values were recorded using an Expert-001 ionomer (*Econix-Expert*, Moscow, Russia)[3] with combined electrodes ESK-10601/7 and ERP-105, whereas the values of *X* were recorded using an Expert-002 conductometer (*Econix-Expert*) with a sensor UEP-P-S operating at a frequency of 1.6 kHz.

After that, we calculated the total degrees of activation (+) or inhibition (−) of the vital activity of test microorganisms by the given concentrations of the tested samples after *k* hours of their joint incubation in a liquid test medium ($\varepsilon_{V,k}$, %) by the following Equation (1):

$$\varepsilon_{V,k} = (\varepsilon_{\mathrm{pH},k} + 0.7\varepsilon_{E,k} + 0.7\varepsilon_{X,k})/2.4 \quad (1)$$

The values of $\varepsilon_{\mathrm{pH},k}$, $\varepsilon_{E,k}$, and $\varepsilon_{X,k}$ were determined separately from pH, *E*, and *X* values of the test media in the MC during the incubation of these MC according to the following Equation (2):

$$\varepsilon_{i,k} = 100 \times (\Delta Yt_{i,k} - \Delta Yc_{i,k})/\Delta Yc_{i,k} \quad (2)$$

The index *i* shows the measurements for which parameter (pH, *E* or *X*) were taken into account in Equation (2): for example, $\varepsilon_{\mathrm{pH},k} = 100 \times (\Delta Yt\mathrm{p}_{\mathrm{H},k} - \Delta Yc_{\mathrm{pH},k})/\Delta Yc_{\mathrm{pH},k}$.

The values of $\Delta Yt_{i,k}$ and $\Delta Yc_{i,k}$ were determined as averaged over a sample of *N* samples with the same concentrations of extracts prepared in the same way from one type of raw material (in our case, $N = 3 \times 4 = 12$), changes in the values of the *i*-parameter of the test medium (pH, *E*, or *X*) that occurred *k* hours after the start of incubation of this medium in the presence of a given concentration of TE (ΔYt observed in the test MC) or in the absence of TE (ΔYc observed in control-1 MC). For example, $\Delta Yt_{\mathrm{pH},2} = \mathrm{pH}_{\mathrm{T},2} - \mathrm{pH}_{\mathrm{T},0}$ and $\Delta Yc_{X,4} = X_{\mathrm{C},4} - X_{\mathrm{C},0}$ (where $\mathrm{pH}_{\mathrm{T},0}$ is the pH value of the medium in the test MC at the beginning of its incubation, $\mathrm{pH}_{\mathrm{T},2}$ is the pH value of the medium in test MC 2 h after the start of its incubation, $X_{\mathrm{C},0}$ is the *X* value of the medium in control-1 MC at the beginning of incubation, and $X_{\mathrm{C},4}$ is the *X* value of the medium in control-1 MC 4 h after the start of incubation).

The error in determining each of the averaged quantities $\varepsilon_{\mathrm{pH},k}$, $\varepsilon_{E,k}$, and $\varepsilon_{X,k}$ was calculated in a standard way [34–36]. On the basis of the standard formula $\Delta z(x_i) = \Sigma_i(\Delta x_i \delta z/\delta x_i)$ [34–36], the total error in determining the value $\varepsilon_{V,k}$ was calculated as $\Delta\varepsilon_{V,k} = (\Delta\varepsilon_{\mathrm{pH},k} + 0.7\Delta\varepsilon_{E,k} + 0.7\Delta\varepsilon_{X,k})/2.4$.

To assess the general degree of activation or inhibition of the vital activity of test microorganisms by the given TE concentrations, we chose the parameters pH, *E*, and *X* because they are most reliably measured instrumentally and, at the same time, sensitive enough to be used in controlling the acceleration or deceleration of conversion by viable microorganisms present in the test medium and catabolites present in the same medium into anabolites after *k* hours of incubation of the mentioned test medium in the presence of TE as compared with the control, in which TE is absent. The sensitivity is due to the transformation of catabolites into anabolites by microorganisms, which significantly changes the acidity, electrochemical redox potential, and electrical conductivity of the test media.

The legitimacy of combining three quantities $\varepsilon_{\mathrm{pH}}$, ε_E, and ε_X into one parameter ε_V can be explained by the fact that each of these quantities was independently normalized to the control values of its defining indicator and, thus, uniformly (percentage relative to the control) reflected the change in metabolism test microorganisms in the presence of TE, at the same time characterizing this change somewhat differently, because changes in pH, *E*, and *X* in the test medium caused different metabolic processes carried out by the presence of viable microorganisms. As a result, the total value of ε_V characterized the changes in the metabolic activity of test microorganisms more informatively and adequately than each of the values of $\varepsilon_{\mathrm{pH}}$, ε_E, and ε_X separately.

The latter is confirmed by the fact that for ε_V there was a 90% significant correlation with the change in the number of colonies forming units of test microorganisms, which is determined using the standard method [1–3, 37, 38].

With the proposed method, it is possible to determine the microbial contamination (C_{M}) of the tested samples. For this, the calculation is performed similarly to Equations (1) and (2), where ΔYt is determined for control-1 MC and ΔYc for control-2 MC. The obtained C_{M} value is multiplied

[3] URL: http://ecosolution.ru. Accessed October 06, 2020.

by the calibration factor, determined previously on the basis of comparing the results obtained using the proposed method with the results obtained for the same concentrations of the same TEs using the aforementioned standard microbiological testing method. In this case, the resulting C_M value will show how many viable microorganisms were initially present in the test sample. Moreover, if the test samples are incubated in selective nutrient media instead of the "general accumulative" nutrient medium used in this study, then the proposed method can be used to determine both the total microbial contamination of these samples and the contamination of individual species and strains of microorganisms.

RESULTS AND DISCUSSION

The table and figure present the most interesting data obtained by the proposed method concerning the objects of this study.

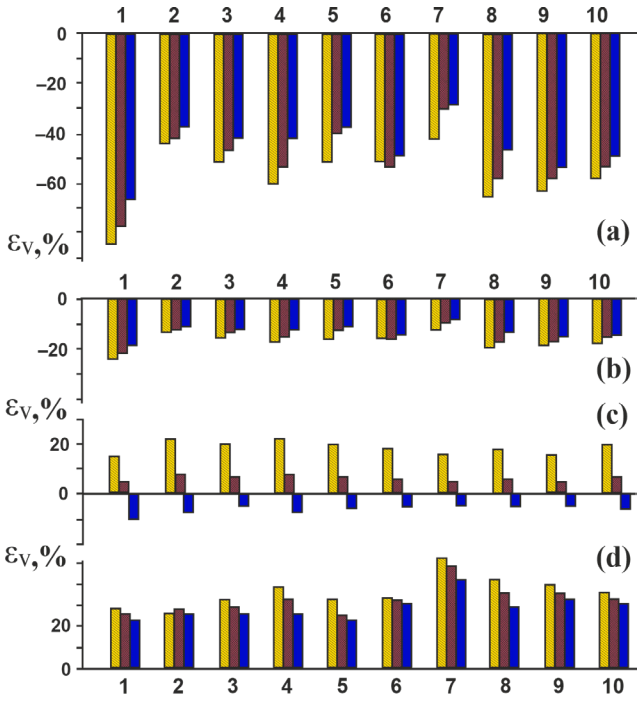
As can be seen in the figure, in the change in the concentration of TEs in the test medium (C_{TE}), the nature of both probiotic and antibiotic activities can change quite significantly.

TEs obtained from different parts of different plants also had different probiotic and antibiotic activities. This is particularly seen in, for example, comparing the antibiotic activity of extracts obtained from the leaves of eucalyptus globular, oregano herb,

The total degree of activation (+) or inhibition (−) of the vital activity of *Lactobacillus acidophilus* ($\varepsilon_{V,k}$, %), determined after 2, 4, and 6 h of their incubation in a liquid nutrient medium in the presence of different amounts of whole subcritical extracts obtained using liquefied CO_2 from various plant materials (CO_2PE)

Biological activity	No. of raw material									
	1	2	3	4	5	6	7	8	9	10
	conc. CO_2PE 3.0 vol %									
$\varepsilon_{V,2}$, %	–84	–44	–51	–60	–51	–51	–42	–65	–63	–58
$\varepsilon_{V,4}$, %	–77	–42	–47	–53	–40	–53	–30	–58	–58	–53
$\varepsilon_{V,6}$, %	–67	–37	–42	–42	–37	–49	–28	–47	–53	–49
	conc. CO_2PE 1.5 vol %									
$\varepsilon_{V,2}$, %	–24	–13	–15	–17	–15	–15	–12	–19	–18	–17
$\varepsilon_{V,4}$, %	–22	–12	–13	–15	–12	–16	–9	–17	–17	–15
$\varepsilon_{V,6}$, %	–19	–11	–12	–12	–11	–14	–8	–13	–15	–14
	conc. CO_2PE 0.5 vol %									
$\varepsilon_{V,2}$, %	15	22	20	22	20	18	16	18	16	20
$\varepsilon_{V,4}$, %	5	8	7	8	7	6	5	6	5	7
$\varepsilon_{V,6}$, %	–10	–7	–5	–7	–6	–5	–4	–5	–5	–6
	conc. CO_2PE 0.2 vol %									
$\varepsilon_{V,2}$, %	28	26	32	38	32	33	52	41	39	36
$\varepsilon_{V,4}$, %	26	28	29	33	25	32	48	36	36	33
$\varepsilon_{V,6}$, %	23	26	26	26	23	31	42	29	33	31

Note. See the Materials and Methods section for the method in determining $\varepsilon_{V,k}$ and the corresponding raw material Nos. 1–10 used to prepare the tested extracts. The relative error in determining ε_V for all values indicated in the table was in the range from 10 to 20%.



Comparative biological activity of the tested extracts (TEs) against *L. acidophilus* at different TE concentrations in the test medium (a: 3.0 vol %; b: 1.5 vol %; c: 0.5 vol %; d: 0.2 vol %). The ordinate shows the ε_V (%) values determined for TE based on the results of measuring the pH, redox potential, and electrical conductivity of liquid nutrient media with *L. acidophilus* after 2, 4, and 6 h of incubation according to Equations (1) and (2). The abscissa shows the number of raw materials from which TEs were obtained.

and meadow mint grass, where at C_{TE} = 3 vol %, the values of $\varepsilon_{V,6}$ were −67 ± 8%, −49 ± 6%, and −28 ± 4% (see table for extracts Nos. 1, 6, and 7). Also, this is seen as well in comparing the probiotic activity of the same extracts, where at C_{TE} = 0.2 vol %, the values of $\varepsilon_{V,6}$ were 23 ± 3%, 31 ± 4%, and 42 ± 5%, respectively).

Among the extracts studied, the most active prolonged (long-term) antibiotic properties (quantitatively characterized in the table by the value of $\varepsilon_{V,6}$, determined after 6 h of incubation of test microorganisms in the presence of TE) were shown by extracts from the leaves of eucalyptus globular and seeds of star anise with $C_{TE} \geq 3$ vol % (see table for extracts Nos. 1 and 9). The most active prolonged probiotic properties were shown by an extract from the herb of meadow mint at C_{TE} = 0.2 vol % (see table for extract No. 7).

In most cases, the initial (short-term) biological activity of TEs (quantitatively characterized in the table by the value of $\varepsilon_{V,2}$, determined after 2 h of incubation of test microorganisms in the presence of TEs) was significantly greater than their prolonged activity. This was probably because of both the adaptation of the test microorganisms to the presence of TE and a decrease in the activity and the total amount of BAS contained in TE per cell of the test microorganism over time. Moreover, the latter took place because the total number of cells of microorganisms increased during incubation of the test medium containing them, whereas the activity and total amount of BAS contained in TE decreased during incubation because of biochemical and physicochemical denaturation and destruction of the mentioned BAS.

The medium-term (in terms of the interaction time of TE with the test microorganisms) biological activity of TE (quantitatively characterized in the table by the value of $\varepsilon_{V,4}$, determined after 4 h of incubation of test media with TE) was intermediate in value between $\varepsilon_{V,2}$ and $\varepsilon_{V,6}$ and only sometimes (as, for example, in the case of extracts Nos. 2 and 9 with $C_{TE} \geq 1.5$ vol %) exceeded both $\varepsilon_{V,2}$ and $\varepsilon_{V,6}$ of the same TE (see table and figure).

The decrease in TE concentrations in the test medium decreased their antibiotic activity against test microorganisms significantly and monotonously and increased, on the contrary, their probiotic activity. For example, at C_{TE} = 3.0, 1.5, and 0.2 vol %, the $\varepsilon_{V,6}$ values for the extract from the leaves of the globular eucalyptus were −67 ± 8%, −19 ± 3%, and 23 ± 3%, respectively, and the values of $\varepsilon_{V,6}$ for the extract from the herb of meadow mint were −28 ± 4%, −8 ± 1%, and 42 ± 5%, respectively (see table for extracts Nos. 1 and 7).

The indicated effective concentrations of TEs turned out to be significantly higher than, for example, the widely used synthetic antiseptic with a broad spectrum of action, such as chlorhexidine bigluconate (CHG), which we studied in the form of a 0.05% aqueous solution manufactured by *Rosbio* (Russia) [29], which already at C_{TE} = 0.0001 and 0.001 vol % showed $\varepsilon_{V,6}$ = −35 ± 5% and −1 ± 6% for *L. acidophilus*. However, CHG is not intended for internal use, and the advantages of PEs over antibiotics have already been discussed above. On the basis of these data, the method of rapid instrumental microbiological testing can be successfully used to assess the probiotic and antibiotic properties of not only PEs but also many other drugs and materials (including those obtained synthetically).

CONCLUSIONS

With the proposed method, it is possible to assess the initial microbial contamination much more quickly (within a few hours, not days), objectively, and informatively than using standard methods, as well as to determine the effect on the dynamics of life

activity of test microorganisms of samples of various products (for example, PEs).

The greater objectivity of the proposed technique is achieved by reducing the role of the subjective human factor when replacing visual methods with instrumental ones in the measurement process. We achieved greater information content of the proposed technique because of the following. Firstly, instrumental measurement methods are more sensitive than visual ones used in standard methods. Secondly, the proposed method makes it possible to assess the dynamics of changes in the vital activity of microorganisms over a set of arbitrarily selected time intervals, in contrast to standard procedures, where measurements are made only once, at the end of the incubation period of the tested samples. Thirdly, the proposed method involves assessing changes in the vital activity of microorganisms at once by several independent indicators (such as pH, redox potential, and electrical conductivity of the test medium), and not just one (turbidity of the test medium, the number of colonies of microorganisms, or the size of the zone of their growth inhibition), as in the case of using standard techniques. The technique presented here uses fewer materials and is less labor-intensive compared with similar standard methods and also provides many more opportunities for automating the entire analysis process.

All this makes the presented technique much more accessible for mass use than the previously used standard methods of microbiological testing and assessment of microbial contamination of samples of various products, which is very relevant because one of the important conditions for ensuring the proper level of safety and quality of life of people is not only timely and qualitative testing of the probiotic and antibiotic properties of new products and individual ingredients and additives to them but also constant wide monitoring of microbial contamination, as well as probiotic and antibiotic properties of products already admitted for mass consumption to identify the poor quality or that had time to deteriorate before the final sale or undergo chemical or biological contamination of its samples.

Regarding the PEs studied, the most active prolonged (long-term) antibiotic properties were exhibited by extracts of eucalyptus spherical leaves and star anise seeds at their concentrations in the test medium from 3 vol % and higher. The most active long-term probiotic properties were exhibited by an extract from the herb of meadow mint at a concentration of 0.2 vol % in the test medium. In most cases, the initial biological activity of the TEs was significantly higher than their long-term activity. The medium-term (in terms of the time of TE interaction with the test microorganisms) biological activity of TEs was intermediate in value and only sometimes exceeded the prolonged and initial biological activity of TEs. With a decrease in the concentration of TEs in the test medium, their antibiotic activity monotonically decreased and their probiotic activity increased.

It is obvious that the nature of the probiotic and antibiotic activities of pharmaceuticals, cosmetics, foods, feeds, and other products, including those containing various PEs, is largely determined by the choice of not only the raw material and the method for extracting BAS from it but also the concentration of active substances in products. Moreover, the exact nature of these dependencies can be established only empirically, with the help of a significant number of tests, which are convenient to carry out using the proposed method.

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Authors' contribution

V.S. Sibirtsev – concept and design of the study, writing the text of the article;

U.Yu. Nechiporenko – collecting materials, conducting research.

The authors declare no conflicts of interest.

REFERENCES

1. Sutherland J., Miles M., Hedderley D., Li J., Devoy S., Sutton K., Lauren D. *In vitro* effects of food extracts on selected probiotic and pathogenic bacteria. *Int. J. Food Sci. Nutr.* 2009;60(8):717-727.
https://doi.org/10.3109/09637480802165650

2. Das S., Anjeza C., Mandal S. Synergistic or additive antimicrobial activities of Indian spice and herbal extracts against pathogenic, probiotic and food–spoiler microorganisms. *Int. Food Res. J.* 2012;19(3):1185-1191.

3. Al-Zubairi A., Al-Mamary M. A., Al-Ghasani E. The antibacterial, antifungal and antioxidant activities of essential oil from different aromatic plants. *Glo. Adv. Res. J. Med. Med. Sci.* 2017;6(9):224-233.
http://garj.org/garjmms

4. Rodino S., Butu M. Herbal Extracts—New Trends in Functional and Medicinal Beverages. In: Grumezescu A.M., Holban A.M. (Eds.). Functional and Medicinal Beverages. Volume 11: The Science of Beverages. Academic Press; 2019. P. 73-108.
https://doi.org/10.1016/B978-0-12-816397-9.00003-0

5. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review. *Int. J. Food Microbiol.* 2004;94(3):223-253.
https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

6. Bakkali F., Averbeck S., Averbeck D., Idaomar M. Biological effects of essential oils – A review. *Food Chem. Toxicol.* 2008;46(2):446-475.
https://doi.org/10.1016/j.fct.2007.09.106

7. Tripathi A.K., Bhoyar P.K., Baheti J.R., Biyani D.M., Khalique M., Kothmire M.S., Bhanarkar A.B. Herbal antidiabetics: a review. *International Journal of Research in Pharmaceutical Sciences (IJRPS).* 2011;2(1):30-37.

8. Fatima A., Alok S., Agarwal P., Singh P.P., Verma A. Benefits of herbal extracts in cosmetics: a review. International *Journal of Pharmaceutical Sciences and Research (IJPSR).* 2013;4(10):3746-3760.
https://doi.org/10.13040/ijpsr.0975-8232.4(10).3746-60

9. Alok S., Jain S.K., Verma A., Kumar M., Mahor A., Sabharwal M. Herbal antioxidant in clinical practice: a review. *Asian Pac. J. Trop. Biomed.* 2014;4(1):78-84.
https://doi.org/10.1016/S2221-1691(14)60213-6

10. Radice M., Manfredini S., Ziosi P., Dissette V., Buso P., Fallacara A., Vertuani S. Herbal extracts, lichens and biomolecules as natural photo-protection alternatives to synthetic UV filters. A systematic review. *Fitoterapia.* 2016;114:144-162.
https://doi.org/10.1016/j.fitote.2016.09.003

11. Merghni A., Marzouki H., Hentati H., Aouni M., Mastouri M. Antibacterial and antibiofilm activities of *Laurus nobilis L.* essential oil against *Staphylococcus aureus* strains associated with oral infections. *Curr. Res. Transl. Med.* 2016;64(1):29-34.
https://doi.org/10.1016/j.patbio.2015.10.003

12. Fani M., Kohanteb J. *In vitro* antimicrobial activity of thymus vulgaris essential oil against major oral pathogens. *J. Evid. Based Complementary Altern. Med.* 2017;22(4):660-666.
https://doi.org/10.1177/2156587217700772

13. Kokina M.S., Frioui M., Shamtsyan M., Sibirtsev V.S., Krasnikova L.V., Konusova V.G., Simbirtsev A.S. Influence of pleurotus ostreatus beta-glucans on the growth and activity of certain lactic acid bacteria. *Sci. Study Res. Chem. Chem. Eng. Biotechnol. Food Ind.* 2018;19(4):465-471.

14. Atarés L., Chiralt A. Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends Food Sci. Technol.* 2016;48:51-62.
https://doi.org/10.1016/j.tifs.2015.12.001

15. Ribeiro-Santos R., Andrade M., Melo N. R., Sanches-Silva A. Use of essential oils in active food packaging: Recent advances and future trends. *Trends Food Sci. Technol.* 2017;61:132-140.
https://doi.org/10.1016/j.tifs.2016.11.021

16. Ju J., Xie Y., Guo Y., Cheng Y., Qian H., Yao W. Application of edible coating with essential oil in food preservation. *Crit. Rev. Food Sci. Nutr.* 2019;59(15):2467-2480.
https://doi.org/10.1080/10408398.2018.1456402

17. Yuan G., Chen X., Li D. Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity, and application in food systems. *Food Res. Int.* 2016;89(1):117-128.
https://doi.org/10.1016/j.foodres.2016.10.004

18. Donsì F., Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. *J. Biotechnol.* 2016;233:106-120.
https://doi.org/10.1016/j.jbiotec.2016.07.005

19. Pavela R., Benelli G. Essential Oils as Ecofriendly Biopesticides? Challenges and Constraints. *Trends Plant Sci.* 2016;21(12):1000-1007.
https://doi.org/10.1016/j.tplants.2016.10.005

20. Rout P.K., Naik S.N., Rao Y.R. Subcritical CO_2 extraction of floral fragrance from Quisqualis indica. *J. Supercrit. Fluids.* 2008;45(2):200-205.
https://doi.org/10.1016/j.supflu.2008.02.011

21. Sahena F., Zaidul I.S.M., Jinap S., Karim A.A., Abbas K.A., Norulaini N.A.N., Omar A.K.M. Application of supercritical CO_2 in lipid extraction – A review. *J. Food Eng.* 2009;95(2):240-253.
https://doi.org/10.1016/j.jfoodeng.2009.06.026

22. Ibadullaeva G.S., Pichkhadze G.M., Ustenova G.O., Dil'barkhanov R., Tikhonova S.A., Grud'ko V.A., Bevz N.Yu., Yudina Yu.V. Chemical composition of the CO_2-extract of *Acorus Calamus* obtained under subcritical conditions. *Pharmaceut. Chem. J.* 2015;49(6):388-392.
https://doi.org/10.1007/s11094-015-1290-0

23. Valle Jr.D.L., Cabrera E.C., Puzon J.J.M., Rivera W.L. Antimicrobial activities of methanol, ethanol and supercritical CO_2 extracts of *Philippine Piper betle L.* on clinical isolates of Gram positive and Gram negative bacteria with transferable multiple drug resistance. *PLoS ONE.* 2016;11(1):e0146349.
https://doi.org/10.1371/journal.pone.0146349

24. Lazarotto M., Valério A., Boligon A., Tres M.V., Scapinello J., Dal Magro J., Oliveira J.V. Chemical composition and antibacterial activity of bergamot peel oil from supercritical CO_2 and compressed propane extraction. *Open Food Sci. J.* 2018;10(1):16-23.
https://doi.org/10.2174/1874256401810010016

25. Vieitez I., Maceiras L., Jachmanián I., Alborés S. Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology. *J. Supercrit. Fluids.* 2018;133(1):58-64.
https://doi.org/10.1016/j.supflu.2017.09.025

26. Coelho J., Veiga J., Karmali A., Nicolai M., Pinto Reis C., Nobre B., Palavra A. Supercritical CO_2 extracts and volatile oil of basil (*Ocimum basilicum L.*) comparison with conventional methods. *Separations.* 2018;5(2):21-33.
https://doi.org/10.3390/separations5020021

27. Sibirtsev V.S. Study of applicability of the bifunctional system "Ethidium bromide + Hoechst-33258" for DNA analysis. *Biochemistry (Moscow).* 2005;70(4.):449-457.
https://doi.org/10.1007/s10541-005-0136-x

28. Sibirtsev V.S. Fluorescent DNA probes: study of mechanisms of changes in spectral properties and features of practical application. *Biochemistry (Moscow).* 2007;72(8):887-900.
https://doi.org/10.1134/S0006297907080111

29. Sibirtsev V.S., Naumov I.A., Kuprina E.E., Olekhnovich R.O. Use of impedance biotesting to assess the actions of pharmaceutical compounds on the growth of microorganisms. *Pharmaceut. Chem. J.* 2016;50(7):481-485.
https://doi.org/10.1007/s11094-016-1473-3

30. Sibirtsev V.S. Biological test methods based on fluorometric genome analysis. *J. Opt. Technol.* 2017;84(11):787-791.
https://doi.org/10.1364/JOT.84.000787

31. Sibirtsev V.S., Maslova A.Yu. Complex research of *E.coli* vital activity dynamics in presence of transition metal ions. *Sci. Tech. J. Inf. Technol. Mech. Opt.* 2019;19(2):236-241.
https://doi.org/10.17586/2226-1494-2019-19-2-236-241

32. Sibirtsev V.S., Uspenskaya M.V., Garabadgiu A.V., Shvets V.I. An integrated method of instrumental microbiotesting of environmental safety of various products, wastes, and territories. *Dokl. Biol. Sci.* 2019;485(1):59-61. https://doi.org/10.1134/S001249661902011X

33. Sibirtsev V.S., Garabadgiu A.V., Shvets V.I. New technique for integrated photofluorescence microbiotesting. *Dokl. Biol. Sci.* 2019;489(6):196-199. https://doi.org/10.1134/S0012496619060103

34. Korn G., Korn T. Mathematical Handbook for Scientists and Engineers. Definitions, Theorems and Formulas for Reference and Review. NY: McGraw Hill Book Company; 1968. 1152 p.

35. Johnson K., Jeffi V. Numerical Methods in Chemistry. Cambridge University Press; 1983. 503 p.

36. Sibirtsev V.S. Analysis of benzo[a]pyrene deactivation mechanisms in rats. *Biochemistry (Moscow).* 2006;71(1):90-98. https://doi.org/10.1134/S0006297906010147

37. Zhuravlev O.E., Voronchikhina L.I. Synthesis and antimicrobial activity of *N*-decylpyridinium salts with inorganic anions. *Pharmaceut. Chem. J.* 2018;52(4):312-315. https://doi.org/10.1007/s11094-018-1813-6

38. Luzhnova S.A., Tyrkov A.G., Gabitova N.M., Yurtaeva E.A. Synthesis and antimicrobial activity of 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones. *Pharmaceut. Chem. J.* 2018;52(6):506-509. https://doi.org/10.1007/s11094-018-1849-7

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