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TECHNOLOGIES FOR OBTAINING CURATIVE AND PREVENTIVE PRODUCTS FROM RAW MATERIALS OF INDIGENOUS VEGETAL ORIGIN

167.01. – BIOTECHNOLOGY, NANOBIOTECHNOLOGY

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The thesis defense will take place on _____, at _____ (a.m./p.m.), in the meeting of the Commission for the public defense of the doctoral thesis, the Polyvalent Hall of the Dimitrie Cantemir State University, 55/4 Hancesti St., Chisinau.

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The summary was sent _____.

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PURPOSE AND OBJECTIVES OF RESEARCH

High-quality curative and preventive medical aid in society is a condition of fruitful economic development by improving the health status of the participants in the economic relations [22]. In this context, the offer of curative-preventive remedies accepted by the population represents enormous advantage, that is why in the last years special attention is paid to the diversification of the range of this type of products, as well as to the study of their mechanisms of action [4,7, 20]. As such, the products that contribute to the correction of adverse alterations in the human body, caused by various agents, some medicinal plants, microalgae and cyanobacteria can be successfully used.

Medicinal plants, microalgae and cyanobacteria enjoy a growing popularity due to their natural origin and their reduced adverse effects or even their absence. Herbal medicines are one of the most popular remedies for prevention, but also for treatment of a large number of diseases such as cardiovascular [1], renal [13, 15], neurological [8, 11, 18], hepatic [25], gynecological [9], cancer [15, 23], metabolic syndrome [15, 17, 24], as well as cellular protectors in the administration of radiotherapy and chemotherapy [10, 14].

Unlike synthetic pharmaceuticals based on unique compounds, which manifest their biological activity independently, many medicinal plants exert their beneficial pharmacological effects through synergistic or additive mechanisms of several active compounds, acting on different targets, associated with a certain pathological mechanism [18]. For example, in case of polycomponent herbal medicines with effects on cancer cells, it was established that the active components affect different phases of the signal transduction ways, including gene expression, cell cycle evolution, proliferation, metabolism and cell apoptosis [12, 17, 21]. Thus, both complex extracts and polycomponent herbal extracts have the ability to act on multiple targets, offering additional preventive and curative benefits.

Among the curative and preventive products from vegetal raw materials, balms are one of the most known and effective forms. The form of hydro-ethanolic extract allows the combination in a single product of the bioactive compounds from 10-40 plant sources, which can ensure a wider spectrum of action and a pronounced efficiency. At the same time, the technologies for the production of balms are quite complicated, starting from the need of combining a large number of components, which must complete each other in such a way as to ensure not only therapeutic efficiency, but also acceptable organoleptic properties. The range of curative-preventive balms is limited, both at the national and international level, with only a few products of this type being

known worldwide - Riga balm, Bittner balm, and at the national level – the balm protected by the patent of invention MD 2103 and produced under the trademark "Fat-Frumos".

The development of new formulas and technologies for obtaining balms based on the vegetal and phytologic material with curative-preventive properties remains a current field of scientific research.

Purpose of the work: development of new technologies for obtaining products with curative-preventive properties - balms - based on the indigenous phytologic raw material and highlighting their biological effects.

Objectives:

- Development of efficient procedures for extracting the active components from the plant and cyanobacterial biomass through environmentally friendly technologies;
- Development of formulas for new curative and preventive products based on plant extracts and cyanobacterial biomass;
- Development of manufacturing technologies of new curative and preventive products;
- Highlighting biological effects of new curative and preventive products.

Research hypothesis. The new curative-preventive balms can be developed on the basis of indigenous plant matter, applying as a criterion of monitoring the antioxidant capacity of the extracts used; the new technologies for the manufacture of balms, based on efficient extraction processes that are environmentally friendly, ensure not only the antioxidant activity of the individual components, but also the manifestation of their synergistic effects when developing balanced recipes for new products.

The obtained result that contributes to solving the important problem of obtaining products of natural origin with curative-preventive properties consists in the scientific foundation of the use as a control criterion of the efficiency of the new products, of their antioxidant capacity, the application of which led to the development recipes and technologies for the production of 6 new balms with determined biological effects, intended for their use in the treatment and prophylaxis of different diseases and pathological conditions.

Know-how and originality of the scientific results: The efficiency of using the values of antioxidant activity as a criterion for evaluating the curative-preventive performances of products based on plant raw materials has been demonstrated. The cumulative / synergistic antioxidant effect of the new curative-preventive formulas obtained from the plant extracts and from spirulina and the pronounced biological effects of the new products have been demonstrated. 6 new formulas and technologies for obtaining curative and preventive products (based on these

formulas) were developed. The know-how and originality of the obtained results is supported by 6 patents for new curative and preventive products, as well as four distinctions at international salons of inventions, offered for these innovations.

Theoretical significance. New data have been accumulated that contribute to complementing the knowledge about the antioxidant activity of the various extracts from plant biomass and about the synergistic effects of the polycomponent natural medications, obtained from several plant sources. The utility of applying the criterion of antioxidant activity in the process of developing curative and preventive products based on plant materials was theoretically substantiated.

Applicative value. The formulas, the technologies of industrial production of 6 new curative and preventive products based on plant extracts and spirulina with pronounced biological effects, without toxicity and adverse effects were developed.

Implementation of results. The technologies for the production of new balms were implemented at the company MAURT based on a license agreement, where experimental parties of the new balms were produced. Also, the results obtained were implemented in the laboratory Food and sanocreatological digestion of the Institute of Physiology and Sanocreatology as elements of nutrition in the development of new food systems.

Publications: 13 publications in total on the topic of the thesis: 5 articles (3 self-authored), of which 2 in journals category B, 1 - category C, 2 in reviewed collections; 2 theses at international conferences, 6 patents.

KEYWORDS

Curative-preventive product, balm, hydro-ethanol extract, macerate, antioxidant activity, biological activity, radioprotective agent, aphrodisiac.

RESEARCH METODOLOGY

Different parts of medicinal plants, extracts from bark and nuts, grape seed extracts, products from the association of heterogeneous crops and medicinal plants AROMED, Republic of Moldova, and cyanobacterial biomass extract *Spirulina platensis* CNMN-CB-11 obtained in the laboratory "Ficobiotehnologie" of the Institute of Microbiology and Biotechnology and at the company FICOTEHFARM SRL, Republic of Moldova were used as plant raw material.

To carry out the present study, a set of methods was used, which can be grouped into three categories: methods for obtaining extracts from plant biomass and spirulina; methods for determining antioxidant activity; methods for testing toxicity and biological effects.

Starting from the nature of the developed products - balms with a volume of at least 40%, hydro-ethanol solutions were used for extractions, with the ethanol concentration starting from 40%. The hydro-ethanol solution is an optimal solvent, as it allows obtaining complex extracts, in which both water-soluble and fat-soluble components are present, thus ensuring a higher biological activity.

As a universal test for assessing the antioxidant capacity in all types of extracts and intermediate and finished products, the $ABTS^{+}$ cation radical reduction test was chosen, as it had positive results in the testing of complex polycomponent hydro, ethanolic and hydro-ethanolic extracts. As what concerns the Spirupotent balm, the NO radical reduction test was also applied, which is significant in case of sexual dysfunctions, as this new product is oriented towards the treatment and prophylaxis of the said dysfunctions. The amount of phenols in spirulina extracts was determined based on the fact that the antioxidant activity of these extracts is largely determined by the phenols. Also, in order to assess the antioxidant activity of spirulina extracts, the capacity of reducing the phospho-molybdenum reagent reagent and DPPH radical was evaluated. [2, 3, 16].

The toxicity tests for the new products were performed according to the national standards in this field, and the biological effects were studied in accordance with the legislation of the Republic of Moldova and the principles of the Declaration of Helsinki.

In order to assess the significance of the differences between the experimental groups, the T Student test was applied, and $P < 0.05$ was considered significant.

SUMMARY OF CHAPTERS

1. NATURAL CURATIVE- PREVENTIVE MEDICINES

The chapter presents a critical analysis of the situation in the field of development and use of herbal and cyanobacterial medicinal medicines. An incursion into the history of phytomedicines is made and different stages and situations of dominance of the idea and implementation of phytotherapy are highlighted. Also, the possible directions of development of this important area are drawn up from the perspective of the development of the contemporary concept of rational phytotherapy and integrative biology.

This chapter discusses the priorities and shortcomings of using single- and polycomponent drug medicines. There are arguments given in favor of herbal medicines, in which, with a very high probability, the synergistic effects of the active substances may be

present. We also reveal the mechanisms by which the natural polycomponent medicines realize their very high therapeutic potential.

An important part of this chapter is dedicated to the technologies for obtaining herbal therapeutic and prevention medicines, but also to the stages, which will be followed in the process of development of a new curative-preventive product. The importance of each stage of work in obtaining a qualitative final result is revealed, so, a high-performance technology for the production of a natural preparation with high efficiency.

The last part of this chapter includes a broad feature of curative-preventive balms, developed and produced in the Republic of Moldova and abroad; and which served as starting points for the research carried out within this doctoral project.

2. OBJECTS AND METHODS APPLIED IN THE RESEARCH

As study objects we described the plant raw materials and cyanobacteria, which were used for the development of the new curative-preventive balms: Liquorice root (*Glycyrrhiza glabra L.*), Sweet Flag rhizomes (*Acorus calamus L.*), the aerial part of perforate St John's-wort (*Hypericum perforatum L.*), the aerial part of Oregano (*Oríganum vulgáre L.*), peppermint leaves (*Mentha piperita L.*), the aerial part of Yarrow (*Achillea millefolium L.*), Pine buds (*Pinus sylvestris L.*), the aerial part of the Imortelle (*Helichrysum italicum* Roth*)*, the aerial part of Lemon Mint (*Monarda citriodora Cerv.ex Lag*), the aerial part of Sage (*Salvia officinalis L.*), Amaranth seeds (*Amaranthus caudatus L.*), the aerial part of Hemp (*Cannabis sativa L.)*, the aerial part and the rhizome of Celery (*Apium graveolens L.*), the root and the aerial part of Parsley (*Petroselinum crispum)*, grape seeds (*Vitis vinifera L.*), walnut shells and septa (*Juglans regia* L.,), Acacia flowers (*Robinia pseudoacacia* L.), produced by the association of ether-bearing crops and medicinal plants AROMED, Republic of Moldova, and biomass of *Spirulina platensis*, obtained in the laboratory "Ficobiotehnologie" of the Institute of Microbiology and Biotechnology and at the company FICOTEHFARM SRL, Republic of Moldova.

The methods applied in this work are described in details. These methods refer to obtaining extracts and macerates from plant biomass and spirulina biomass, determining the antioxidant activity of individual extracts and mixtures and assessing the biological effects exerted by new curative-preventive balms.

Extracts from plant biomass and spirulina were obtained by the simple extraction procedure with hydro-ethanol solution with variable duration of contact of the biomass with the solvent, by continuous stirring, or by maceration and re-maceration (fractionated maceration).

5 tests were performed to determine the antioxidant activity of new extracts, macerates and new products, which allow revealing the ability of the raw materials to give the antioxidant protection through different mechanisms of action.

The toxicity study of the components of the curative-preventive medicines and of the balm variants was performed according to the international recommendations ICH M3 (R2), and included the analysis of the physiological, hematological, biochemical and morphological parameters of the laboratory animals.

The biological effects of the prepared balms were studied in accordance with the laws of the Republic of Moldova and taking into account the principles of international law and the Declaration of Helsinki. In order to assess the significance of the differences between the experimental groups, the T Student test was applied, and $P < 0.05$ was considered significant.

3. METHODS FOR OBTAINING EXTRACTS WITH ANTIOXIDANT PROPERTIES FROM VEGETAL RAW MATERIALS

In many pathophysiological conditions, including inflammation, neurodegenerative diseases and cancer, reactive oxygen and nitrogen species are involved. Accumulative evidence shows that oxidative impairment of biomolecules including lipids, proteins and DNA contributes to the appearance and evolution of these diseases [19]. Plants contain several groups of compounds with antioxidant efficacy, such as vitamins E and C, polyphenols, flavonoids, carotenoid pigments and anthocyanins. In small amounts, antioxidants significantly inhibit oxidation reactions of lipids, DNA and proteins, which normalizes immune reactions and reduces pathological metamorphosis of cells [7]. In this context, the antioxidant properties of different herbal remedies can largely ensure their benefits in the prophylaxis and treatment of various diseases associated with oxidative stress.

Given their important health effects, the methods of efficient extraction of natural antioxidants, the proper evaluations of antioxidant activity, as well as the main resources in the category of plants and cyanobacteria, attract the special attention of researchers in the field of pharmaceuticals and medicine [3, 5, 6, 19]. In order to improve the extraction of antioxidant components from plant materials, it is important to develop new, environmentally friendly processes to reduce the operational time and downgrade the level of use of toxic organic solvents. As a control parameter in the process of development of the procedures for obtaining extracts from plant biomass and that of spirulina, their antioxidant activity was selected.

3.1. Methods for extracting antioxidant components from plant biomass

The most commonly applied processes for obtaining the components of plant biomass are extraction with different solvents, extraction in supercritical CO_2, maceration, re-maceration, and decoctions. Starting from our aim to obtain curative and preventive products such as balms, it is advisable to use extracts or macerates from the biomass of the plant species selected for use, and as a solvent, such as ethanol or hydro-ethanol solutions of different concentration. In order to reveal the best process of extraction in solution of the components with antioxidant action, optimizations were performed on two variables - the concentration of ethanol and the contact time of the solvent with the vegetal biomass. As a unique method of determining the antioxidant capacity of the obtained components, the method for reducing the $ABTS^{\cdot+}$ cation radical was selected, which is appreciated for the possibility of being applied to complicated mixtures of different active substances with different degrees of polarity.

In the first stage, the antioxidant activity of the extracts from the plant biomass, selected as the main components of the balms was tested, by of applying different concentrations of ethanol. The obtained results are shown in Figure 1.

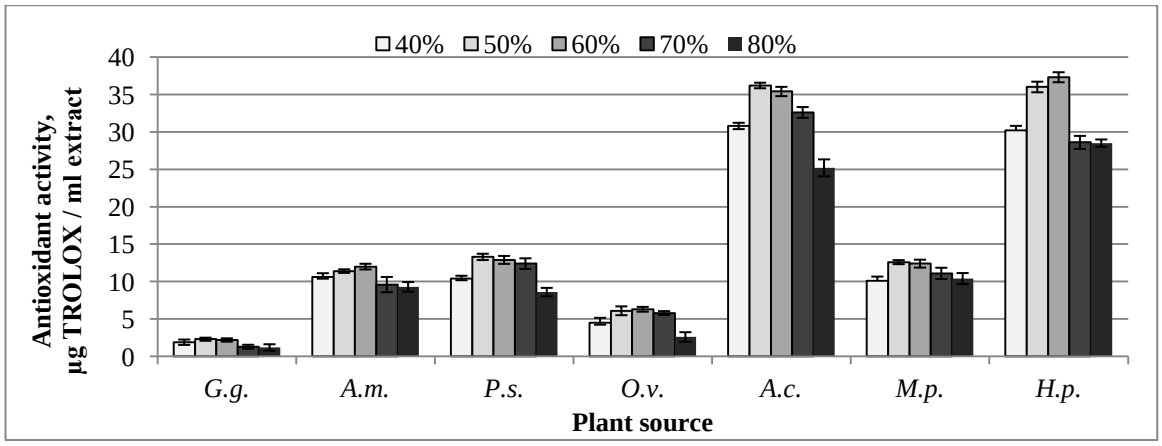

Fig.1. Antioxidant activity (expressed in µg TROLOX in ml of extract) in the hydro-ethanolic extracts from the biomass of the basic components of the new products. *G.g* - *Glycyrrhiza glabra L., A.m. - Achillea millefolium L., P.s. - Pinus sylvestris L.,O.v.* - Oríganum vulgáre L., *A.c. - Acorus calamus L., M.p.* - Mentha piperita L., *H.p.* - *Hypericum perforatum L.(mass ratio 1:10, extraction time -60 min.)*

The most active extracts are obtained in concentrations of 50, 60 and 70% ethanol. In most cases, the difference between these variants is insignificant, so that the concentration of the 50% hydro-ethanol solution is considered as optimal. The highest antioxidant activity among the basic components is extracted from the Sweet Flag rhizomes (25,2-36,2 µg Trolox / ml of extract) and aerial parts of perforate St John's-wort (28,5-37,3 µg Trolox / ml of extract).

The extracts obtained were also tested in terms of the ability to reduce the nitric oxide radical. The results are presented in Figure 2. In most cases, the main factor of variation in the capacity to reduce the nitric oxide radical was the plant species used for extraction, and the activity of the extracts obtained with hydro-ethanol solutions of different concentrations was quite homogeneous in each type of raw material.

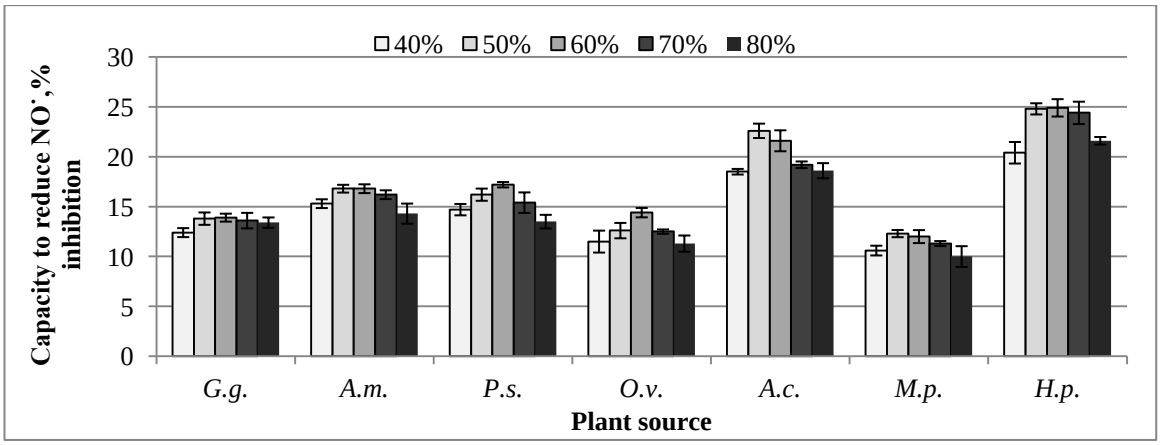

Fig. 2. The ability to reduce the nitric oxide radical (expressed as% inhibition) of the hydro-ethanol extracts from the biomass of the basic components of the new products. ***G.g - Glycyrrhiza glabra L., A.m. - Achillea millefolium L., P.s. - Pinus sylvestris L.,O.v. - Origanum vulgare L., A.c. - Acorus calamus L., M.p. - Mentha piperita L., H.p. - Hypericum perforatum L.(mass ratio 1:10, extraction time -60 min.)***

The same type of extracts was made from the biomass of the additional components of the new products. The results obtained are presented in Figure 3. As with the basic components of the new medicines, the tested extracts had the highest antioxidant activity when applying the ethanol concentration of 50-80%, and the concentration of 50% ethanol was selected as the optimum concentration. The highest antioxidant activity was recorded in the extracts obtained with 50% hydro-ethanol solution from the aerial part of parsley (33.7 ± 0.34 μg Trolox / ml) and sage (38.4 ± 0.94 μg Trolox / ml). The highest capacity to reduce the nitric oxide radical is extracted from the aerial parts of sage and parsley, with values ranging from 17.3 to 23.4% of inhibition. In the case of extracts from the aerial parts of parsley and sage, as well as those from amaranth seeds, the activity of the extracts obtained with 50% hydro-ethanol solution is significantly higher compared to that of the extracts obtained with 40% ethanol solution ($P < 0.005$). No significant differences were found between the activity of 50% and 60% extracts. Thus, the optimum concentration of the hydro-ethanol solution applied to obtain extracts with a high capacity to reduce the nitric oxide radical is 50%.

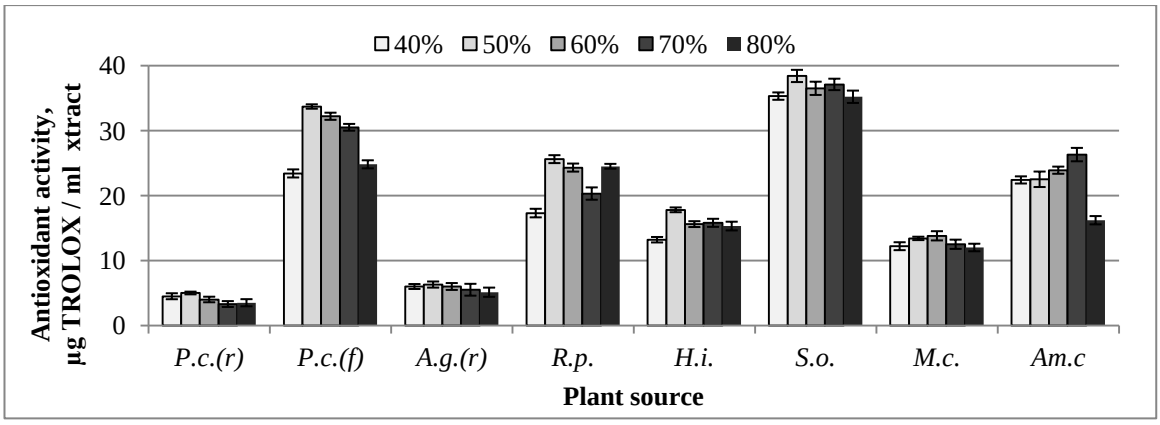

Fig. 3. Antioxidant activity (expressed in µg TROLOX in ml of extract) in the hydro - ethanol extracts from the biomass of the additional components of the new products. *P.c.(r) –Petroselinum crispum L., P.c. root (f)* - aerial part of *Petroselinum crispum L., A.g. (r) – Apium graveolens L, R.p. root - Robinia pseudoacacia L., H.i. - Helichrysum italicum* Roth., *S.o. – salvia oficinalis L., M.c. - Monarda citriodora Cerv.ex Lag, Am.c. - Amaranthus caudatus L.(mass ratio 1:10, extraction time -60 min.)*

Extraction time is the second variable verified in this study. Thus, extraction from the biomass of the basic components with a 50% ethanol solution was achieved, and the contact time was gradually increased up to 180 min. The results obtained are shown in figure 4. The increase of the contact time of the biomass with the hydro-ethanol solution leads to an increase of the antioxidant activity in the extract. Thus, the values obtained when applying an extraction of 180 minutes are significantly higher compared to those obtained in the extractions of 60 minutes ($P < 0.01$) for all the components except the extract from *Origanum vulgare*, where this difference is statistically insignificant.

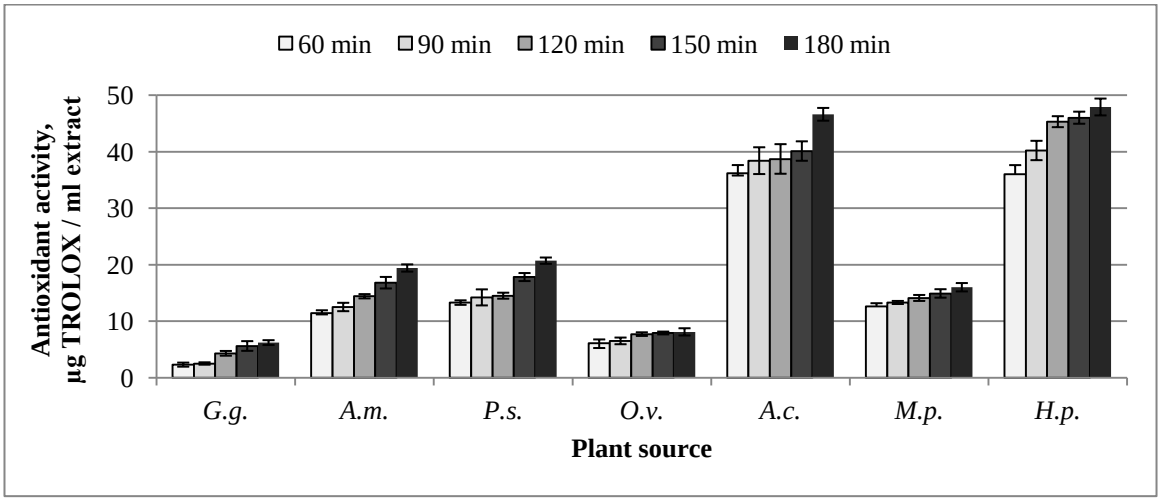

Fig. 2.3. Antioxidant activity (expressed in µg TROLOX in ml of extract) in the hydro-ethanolic extracts (50%) from the biomass of the basic components of the new products depending on the time. *G.g - Glycyrrhiza glabra L., A.m. - Achillea millefolium L., P.s. - Pinus sylvestris L.,O.v.* - Oríganum vulgáre L., *A.c. - Acorus calamus L., M.p.* - Mentha piperita L., *H.p. - Hypericum perforatum L.*

For the additional components of the balms as well as for the ability to reduce the nitric oxide radical, the results show the same tendency: higher activity is characteristic for the 50-60% hydro-ethanol extracts obtained with 180 minutes contact.

The efficiency of the 3-hour extraction was compared with the efficiency of single and triple maceration of the same type of biomass for all basic and auxiliary components. The results for the basic components are shown in Figure 5. The application of the maceration procedure for 5 days, as described in Chapter 2, leads to a significant increase of the antioxidant activity of the obtained extracts. The perforate St John's-wort macerate is in 1.27 times more active as the simple extract, and the macerate from the Sweet Flag rhizomes is in 1.76 times more active as the hydro-ethanolic extract. The macerates from the aerial part of Yarrow, Peppermint and pine buds are 2.1, 2.4 and 2.5 times more active than the respective extracts. An even more significant increase of the antioxidant activity of the macerate in relation to the extract is observed in the Oregano and Liquorice root - 3.2 and 4.1 times respectively. The application of the re-maceration procedure (triple maceration) leads to a further increase of the antioxidant activity of the extraction product - by 10.1 - 48.7% compared to the simple maceration. The same was observed in the additional components.

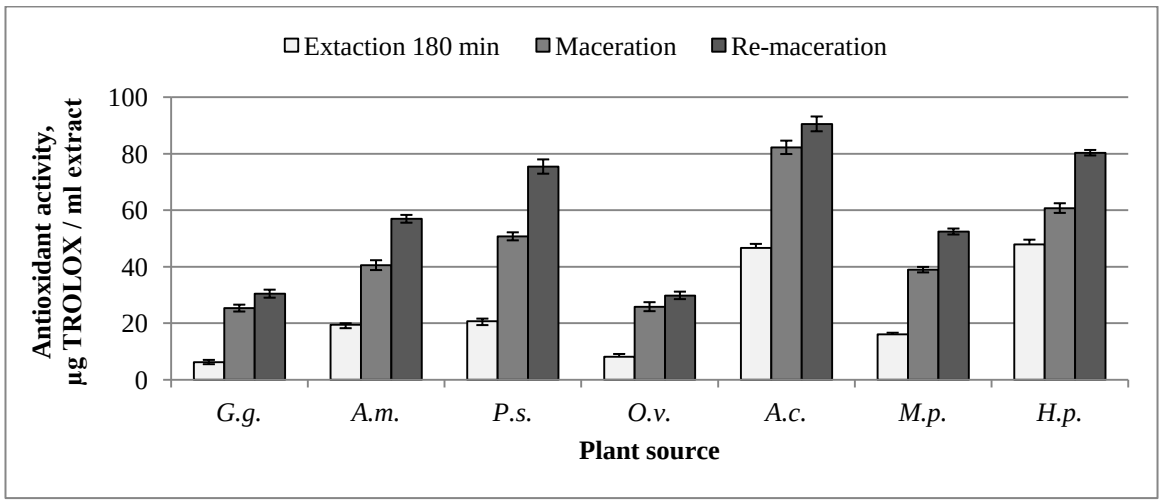

Fig. 5. Antioxidant activity (expressed in µg TROLOX in ml of extract) in the hydro - ethanol extracts (50%), macerated and re-macerated from the biomass of the basic components of the new products. *G.g* - *Glycyrrhiza glabra L.*, *A.m.* - *Achillea millefolium L.*, *P.s.* - *Pinus sylvestris L.*,*O.v.* - Oríganum vulgáre L., *A.c.* - *Acorus calamus L.*, *M.p.* - Mentha piperita L., *H.p.* - *Hypericum perforatum L.*

Alongside with the re-macerates of the separate basic components, a re-macerate of the mixture made up of equivalent masses of these components was made. The antioxidant activity of the re-macerate in the mixture was found to be over 13 times more active than the most active individual extract of the Sweet Flag rhizome. The result is shown in Figure 6.

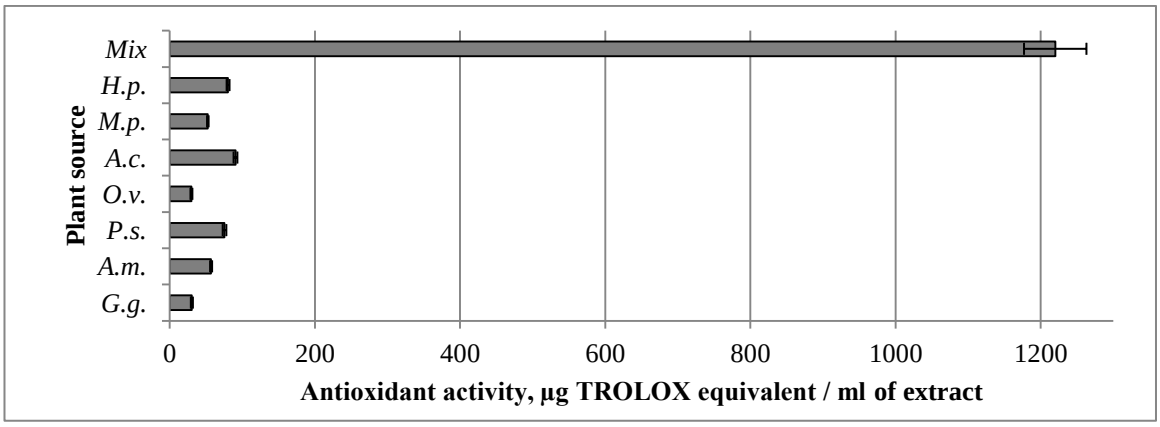

Fig.6. Antioxidant activity of individual re-macerates and re-macerat from the mixture of basic components in the new balms.

Thus, the obtained results suggest that, in order to obtain curative and preventive products with high antioxidant activity, the mixed plant components should be subjected to the re-maceration process (triple maceration). This process, shown in figure 7, allows obtaining an antioxidant activity that is significantly higher than the activity of the individual components of the mixture.

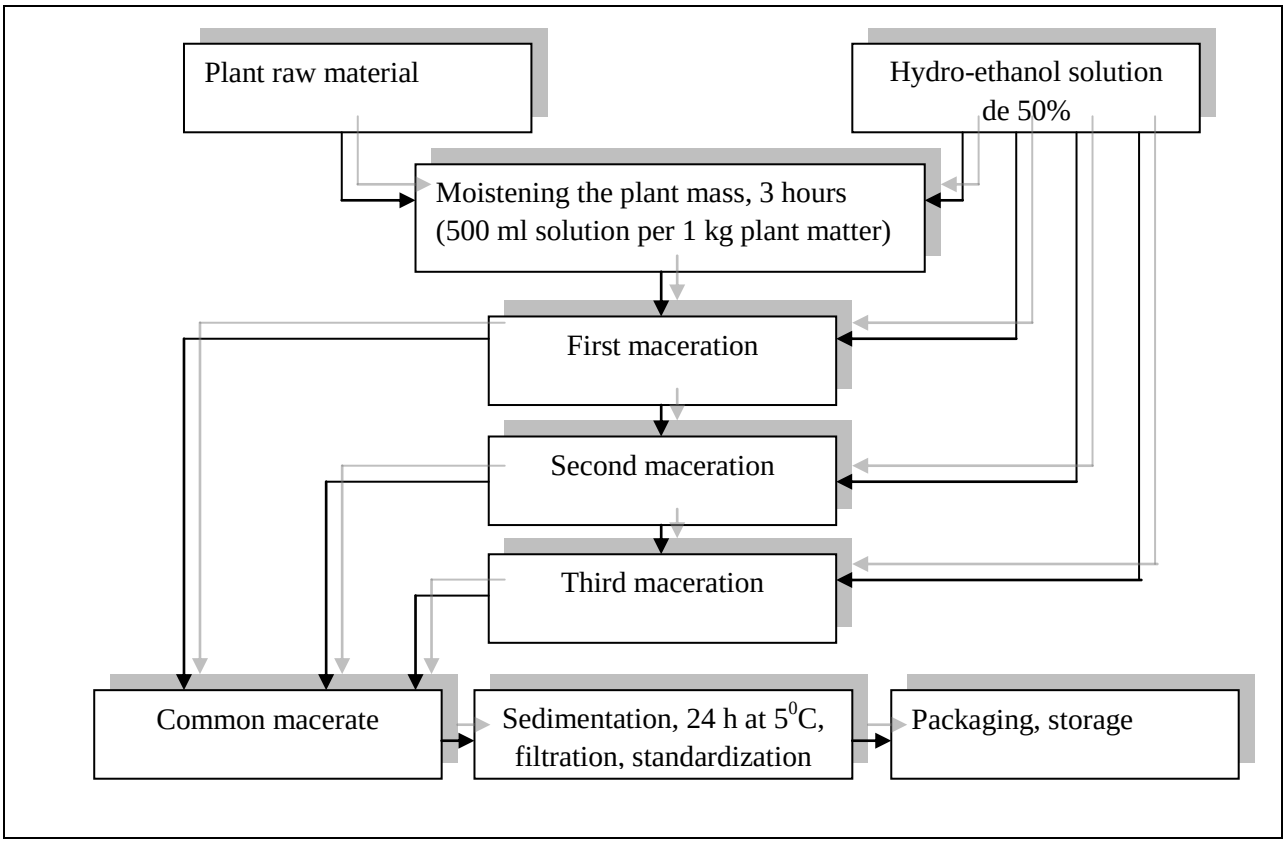

Fig.7. Method for obtaining macerate from plant raw material

Thus, the application of triple maceration with 50% hydro-ethanol solution for the extraction of the antioxidant components from the plant raw material ensures the extraction with the highest antioxidant capacity.

3.2. Methods for extracting antioxidant components from spirulina biomass

The first stage of the research consisted in selecting the optimal parameters (ethanol concentration and contact time) for extracting the components with antioxidant action. The monitoring parameters were the following: the capacity to reduce the DPPH radical and the phospho-molybdenum reagent, as well as the amount of phenols. The optimization of the extraction procedure was performed by applying a factorial experience with two factors, realized in 3 variants according to the 3 monitored parameters. The highest reduction capacity of the phospho-molybdenum reagent, of 87.14 mg equivalent ascorbic acid/g, was obtained in the case of 70% solution and the extraction duration of 90 minutes. The highest antiradical ability (48.5% $DPPH^{\cdot}$ inhibition) was obtained when using a 76% solution and 100 min extraction time, and the highest amount of phenols (7.41 mg Gallic acid equivalent/g) was obtained at the extraction with 70% solution within a period of 100 min.

Given the fact that, with reference to this section of the study, our aim was to reveal a unique solution for obtaining an extract with high activity of spirulina, it was decided to proceed with the method of a single extraction, and to establish the value of the variables, an experiment with 5 extraction variants (experience step for ethanol concentration was 15%, and for extraction time - 10 min). Thus, the tested variants were as follows: 40% ethanol - 70 minutes; 55% ethanol - 80 min; 70% ethanol - 90 min; 85% ethanol - 100 min; 96% ethanol - 110 min. The results for the three tests are shown in Figure 8.

The highest level of antioxidant activity was 88.2 mg of ascorbic acid equivalent/g of dry substance. It is obtained under by extracting with 70% hydro-ethanol solution within 90 min. Under the same experimental conditions we have also obtained the highest level of antiradical activity - 49.3% inhibition of the $ABTS^{\cdot+}$ cation radical and the highest level of phenols in extract - 7.6 mg equivalent Gallic acid / g of dry matter. Thus, the investigations carried out resulted in the development of the method of obtaining the preparation of spirulina with antioxidant properties, which can be seen in figure 9.

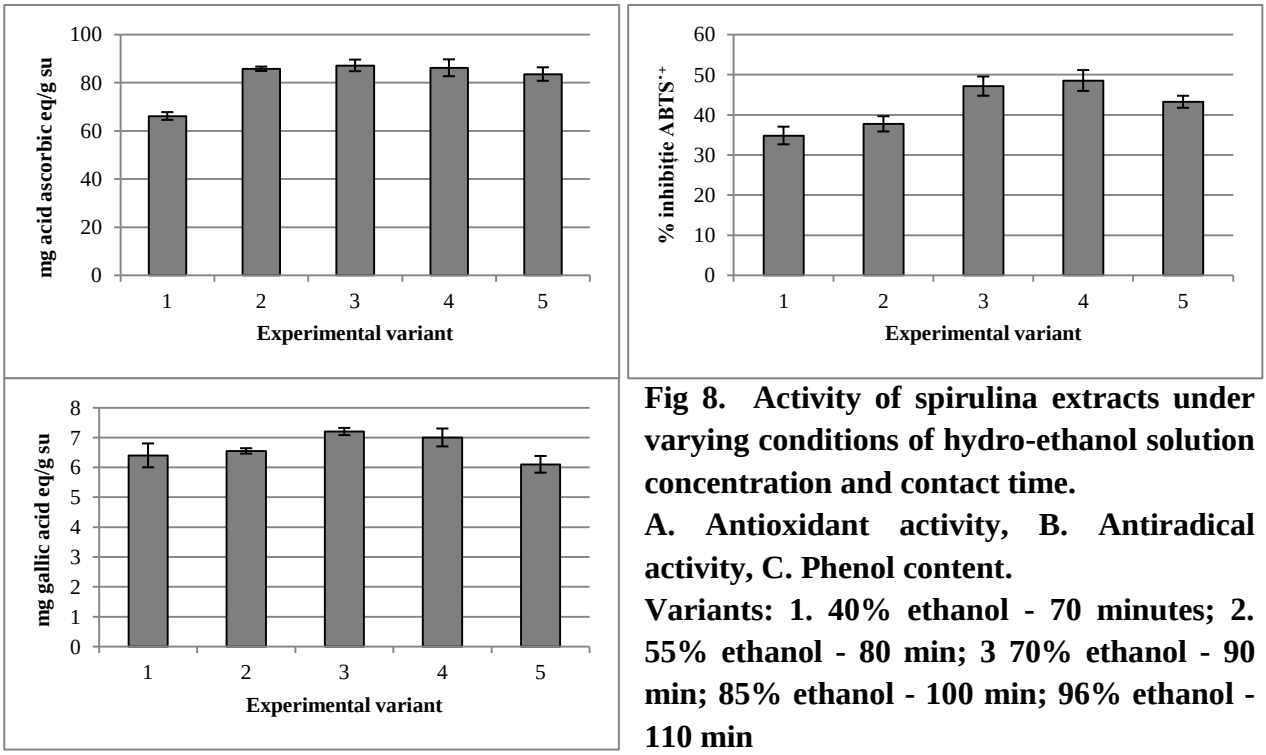

Fig 8. Activity of spirulina extracts under varying conditions of hydro-ethanol solution concentration and contact time.
A. Antioxidant activity, B. Antiradical activity, C. Phenol content.
Variants: 1. 40% ethanol - 70 minutes; 2. 55% ethanol - 80 min; 3 70% ethanol - 90 min; 85% ethanol - 100 min; 96% ethanol - 110 min

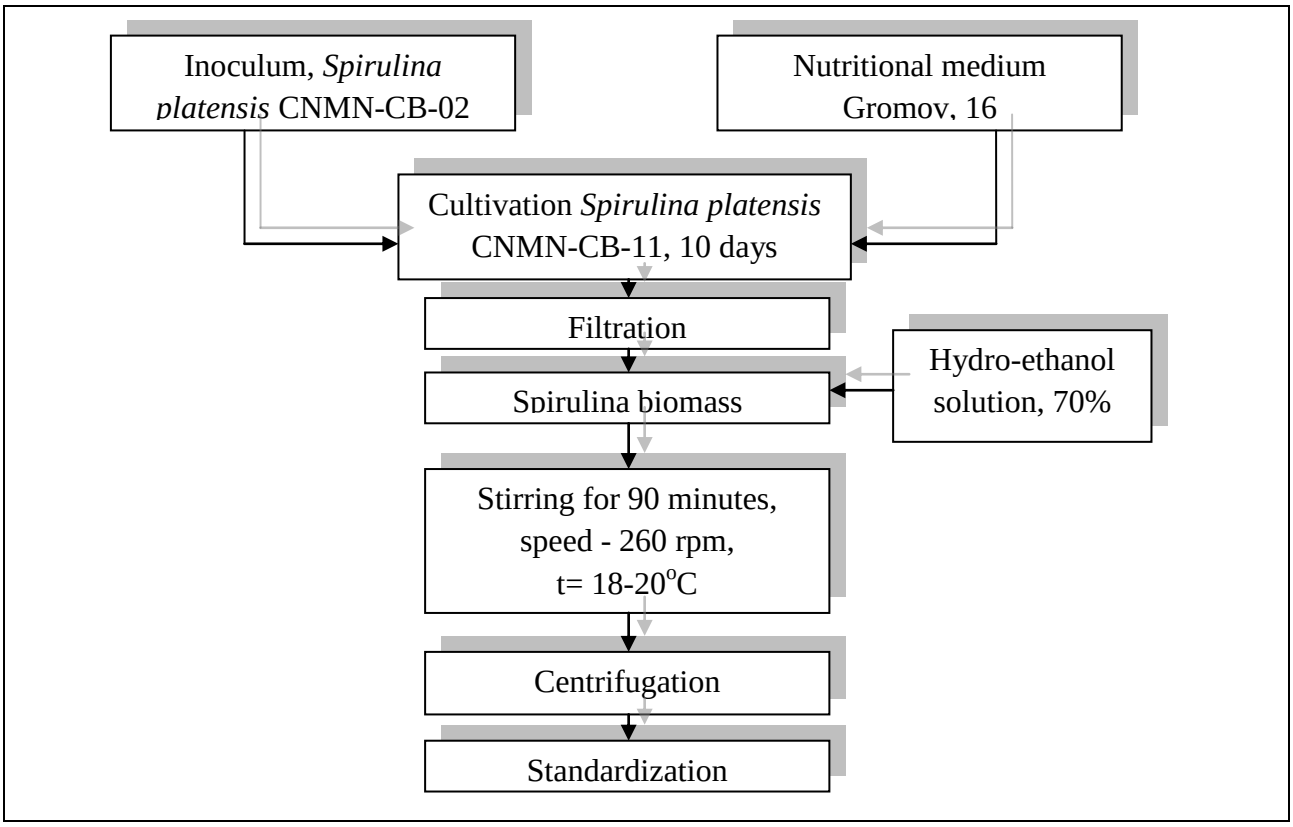

Fig.9. Method for obtaining the active extract of spirulina biomass

4. DEVELOPMENT OF TECHNOLOGIES FOR MANUFACTURING NEW CURATIVE- PREVENTIVE PRODUCTS

4.1. Development of new curative-preventive balm recipes

Based on the bibliographic study and the own results obtained in the process of testing different plant materials, 6 new recipes were developed for the manufacture of 5 curative - preventive balms based on plant raw materials, with the common name Fat-Frumos and one from plant matter and spirulina - Spirupotent (trademarks registered by the author).

The main elements of the new recipes are the basic plant components. In 5 of the 6 new conditioners (Spirupotent, and 4 of the Fat-Frumos group - B_1368, B_1369, B_1370, B_1377) the same basic components were used, namely Liquorice root, Sweet Flag rhizome, the aerial part of perforate St John's-wort, the aerial part of Oregano, Peppermint leaves, the aerial part of Yarrow, pine buds, and for the balm B_1378 – Liquorice root, the aerial part of the Oregano, Peppermint leaves and pine buds.

The following components were used in the Spirupotent balm as additional components: Acacia flowers, rhizomes and aerial part of celery, root and aerial part of parsley, walnut shells and septa, grape seeds and standardized spirulina extract. The last three components are not subject to maceration. In the composition of the balms are used the extracts from the manufacturer, or prepared by simple hydro-ethanol extraction, according to the specific prescriptions. The recipes of the new balms are presented in Table 1.

4.2. Development of the manufacturing technology of the new curative-preventive balms

In the development of the production technologies of new curative and preventive products, the specific activity of the ingredients was taken into account, so that the finished product is characterized by maximum biological activity. Further, the production technologies of the 6 new balms and the test results in the critical points of the technological process are described, which demonstrate the correctness of applying different elements of the production flow. Achieving the ultimate goal of these technologies - obtaining products with valuable organoleptic properties and high biological activity - is demonstrated, along with other important characteristics, by the high values of the antioxidant activity of the balms and the maintenance of the activity level during the technological flow. The technological process for the manufacture of Spirupotent balm is shown in figure 10.

Table 1. The composition of the new curative-preventive balms

Components of the balm	Balm					
	Spirupo-tent	Fat –Frumos				
		B_1368	B_1369	B_1370	B_1377	B_1378
g/L						
Liquorice root	1,5-2,5	3,5-4,5	3,5-4,5	3,5-4,5	3,5-4,5	3,5-4,5
Sweet flag rhizome	0,10-0,16	0,10-0,16	0,10-0,16	0,10-0,16	0,10-0,16	
Aerial part of perforate St John's-wort	0,6-0,7	0,6-0,7	0,6-0,7	0,6-0,7	0,6-0,7	
Aerial part of Oregano	0,49-0,55	0,49-0,55	0,49-0,55	0,49-0,55	0,49-0,55	0,49-0,55
Peppermint leaves	0,78-0,84	0,78-0,84	0,78-0,84	0,78-0,84	0,78-0,84	0,78-0,84
Aerial part of Yarrow	0,70-0,80	0,70-0,80	0,70-0,80	0,70-0,80	0,70-0,80	
Pine buds	0,8-1,2	0,8-1,2	0,8-1,2	0,8-1,2	0,8-1,2	0,8-1,2
Amaranth seeds				1,5-2,5	1,5-2,5	1,5-2,5
Hemp extract				1,5-2,5	1,5-2,5	1,5-2,5
Aerial part of the Immortelle		1,5-2,5			1,5-2,5	1,5-2,5
Decorative mint		1,5-2,5			1,5-2,5	1,5-2,5
Sage		1,5-2,5			1,5-2,5	1,5-2,5
Acacia flowers	1,5-2,5					
Rhizomes and aerial part of celery	4,5-5,5					
Parsley root and leaves	1,5-2,5					
Caramel		8,5-11,5	8,5-11,5	8,5-11,5	8,5-11,5	8,5-11,5
Citric acid		0,07-0,11	0,07-0,11	0,07-0,11	0,07-0,11	0,07-0,11
Vanillin		0,21-0,29	0,21-0,29	0,21-0,29	0,21-0,29	0,21-0,29
Dihydroquercetin			1,2			
Lecithin			1			
glycine			30			
ml/L						
Nut shells, 10 mg extract / ml	0,05-0,1					
Grape seeds, 0.05 mg / l extract	1,5-2,5					
Red wine	300-400	300-400	300-400	300-400	300-400	300-400
Ethyl alcohol	285-289	285-289	285-289	285-289	285-289	285-289
Spirulina platensis, 20 mg / ml extract	0,05-0,1					

At this stage samples were taken for research purposes after sedimentation in which the antioxidant activity and the capacity to reduce the nitric oxide radical were determined (the results are presented in Table 2).

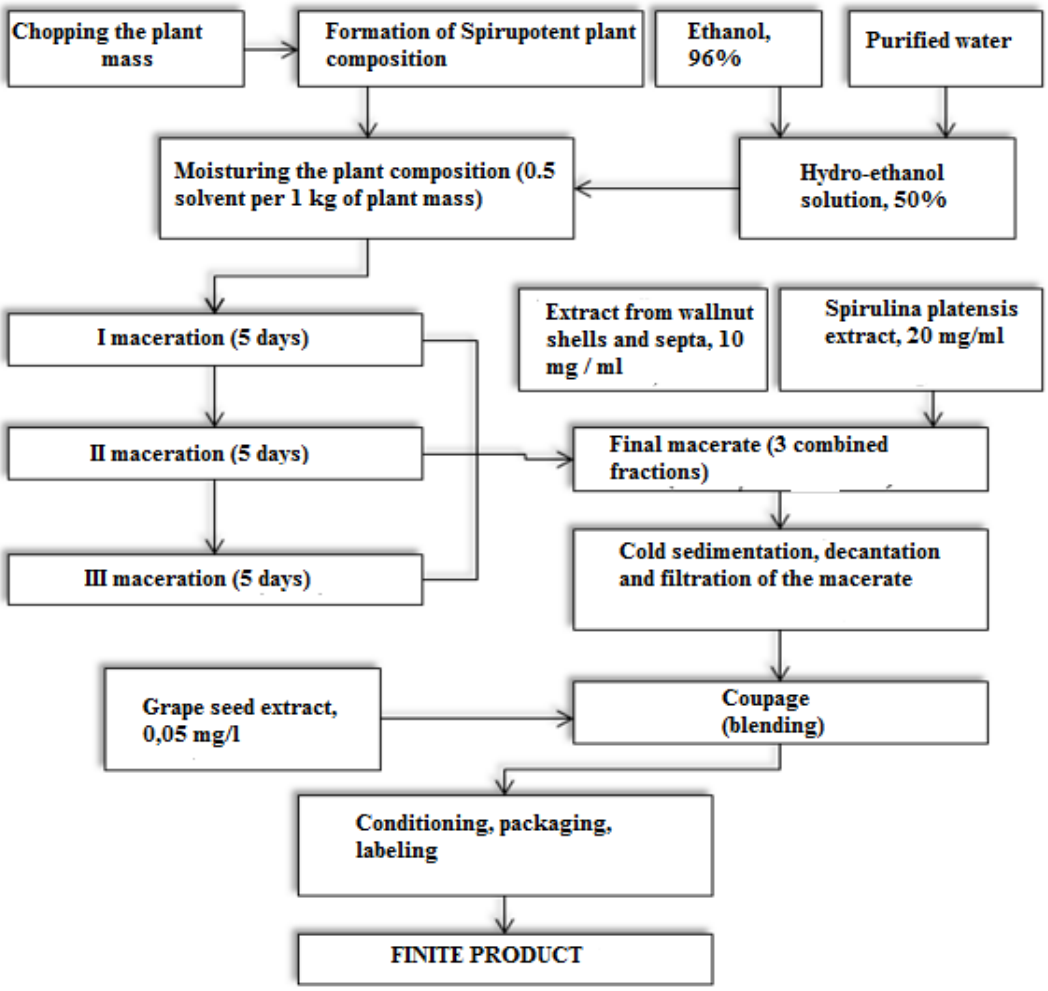

Fig. 10. Technological process of industrial production of Spirupotent balm

Table 2. The ability to reduce radicals $ABTS^{+\cdot}$ and $NO^{\cdot}$ of the components of the Spirupotent balm at different stages of the technological flow

Composition of the balm	Technological flow stage	Antioxidant activity	
		Reduction capacity of $ABTS^{+\cdot}$ µg Trolox equivalent / ml	Reduction capacity of $NO^{\cdot}$ % inhibition
Macerate	I maceration	968±44	62,5±1,8
	II maceration	549±24	44,8±0,9
	III maceration	360±27	40,6±2,5
Combined extract	After unification of the 3 fractions	1220±43	65,2±0,9
Spirulina platensis (extract 20 mg/ml)	Before added	88,2±2,4	43,0±0,4
Juglans regia (extract 10 mg/ml)	Before added	98,4±1,79	28,6±1,28
Vitis vinifera (extract 0,05 mg/l)	Before added	125,6±4,85	41,6±2,11
Red wine	Before added	1644±51	82,5±2,0
Spirupotent Balm	Finished product	5678±62	94,4±1,7

The results show a high capacity of the finished product Spirupotent balm to reduce the $ABTS^{+\cdot}$ cation radical and nitric oxide radical. It is important to mention that the balm production technology ensures a combination of components, which are characterized by synergism of the components with respect to the tested radicals.

The technologies developed for Fat-Frumos series balms were tested according to the same principles and demonstrated the synergistic character of the interaction of their components. All new balms, obtained by applying the technologies described, are characterized by high antioxidant activity. Further addition of the treated red wine and additional components, such as vanillin, citric acid, dihydroquercetin, glycine, lecithin, leads to intensified manifestation of antioxidant effects of the finite product compared to the activity of the macerates obtained from the vegetal mix used in the manufacture of each type of balm. Figure 11 shows the antioxidant activity of triple macerates from plant mixtures compared to the activity of the finished product.

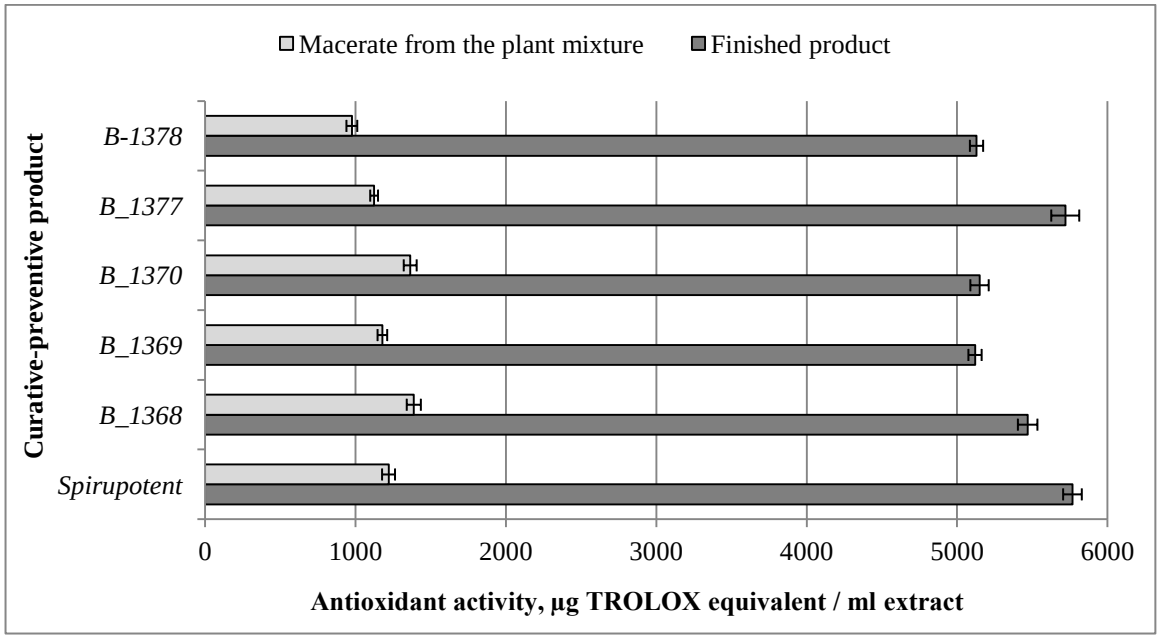

Fig.11. Antioxidant activity of finished curative and preventive products compared to the activity of macerated extract from vegetal mixtures according to manufacturing recipes

Thus, the developed technologies allow obtaining products with high antioxidant properties, which exceed the macerate activity of the six basic components by 3.78 - 5.26 times, and that of the individual components - by 63-190 times. Balms have improved organoleptic properties, are sugar-free, do not contain synthetic preservatives. These technologies allow widening the assortment of natural curative and preventive products, obtained from plant and cyanobacterial biomass.

5. BIOLOGICAL EFFECTS OF NEW CURATIVE AND PREVENTIVE PRODUCTS

The toxicity and biomedical tests, the results of which are presented in this paper were performed on the new curative-preventive balm Spirupotent and on the balm B_1377.

Also, the results obtained in the clinical testing of balm B_1377 are presented

5.1. Toxicity of new curative and preventive products

Acute toxicity tests were performed on rats and mice by administering balm doses of 30, 40, 50 and 60 ml/kg, in parallel with the 42% alcohol toxicity test. The data presented attest that the lethal dose of Spirupotent and B_1377 balm for 100% of animals constitutes: 60 ml/kg for rats and 45 ml/kg - for mice. The 42% ethanol toxicity constitutes 50 ml/kg and 45 ml/kg, respectively. The half-lethal dose of DL_{50} of the 1377 balm for rats is 45.7 ± 1.5 ml/kg, for mice - 30.8 ± 1.4 ml / kg. DL_{50} LD50 of 42% ethyl alcohol solution constituted: 40.5 ± 1.3 ml / kg - for rats and 24.0 ± 1.1 ml/kg - for mice. Thus, the results show that the toxicity of balm is defined by the toxicity of the ethyl alcohol in its composition, and the macerates of medicinal plants reduce its toxicity by up to 28%. The chronic toxicity study of balm 1377 in mice lasted four months, the balm being administered daily in doses of 1%, 2% and 5% of LD_{50}. The rats were given the same doses and the test duration was 6 months. During the experiments, no toxic effects were recorded on the animals.

5.2. Biological effects of new curative-preventive balms in standard conditions and the action of ionizing radiation

Treatment of laboratory animals with the radioactive isotope Cesium^{137}Cs for 15 days results in disorders of the body's functions - reduction of coagulation time, increased alanine transaminase activity, occurrence of perivascular edema in brain tissue, vacuolization of hepatocyte cytoplasm. The administration of the herbal balm based on macerates results in the improvement of the displaced hematological indices (Table 3).

Also, the balm contributes to the normalization of the hepatic indices, the complete disappearance of the alterations in the subcellular structures of the liver, the reduction of the destructive lesions in the organs affected by the action of the radioactive isotope. Additionally, the balm permanently removes the negative changes in the superior nervous activity, exerts a toning effect, and reduces the duration of induced sleep by 10%.

Table 3. Influence of Spirupotent balm on blood coagulation time and amount of alanine transaminase in rats

Group of animals	Observation term	
	Before administration	After 15 days of administration
blood coagulation time in rats (seconds)		
Witness	101,0±4,6	102,3±5,4
Balm	105,5±5,1	96,4±6,1
Alcohol	102,4±5,8	115,2±5,3
Cesium-137	106,1±7,4	41,8±4,1*
Cesium-137 and balm	105,7±6,2	97,5±5,6
Cesium-137 and alcohol	102,5±6,3	49,4±6,1*
Alanine transaminase, mM/l		
Witness	1,55±0,11	1,58±0,09
Balm	1,51±0,12	1,52±0,06
Alcohol	1,49±0,24	1,61±0,22
Cesium-137	1,51±0,15	2,04±0,04*
Cesium-137 and balm	1,52±0,08	1,50±0,14
Cesium-137 and alcohol	1,52±0,15	1,90±0,11*

5.3.Biological effects of balms on cancer of the reproductive organs and mammary glands and sexual dysfunctions

To support the immunostimulatory, detoxifying, cytolytic and cholestatic effects, a study was performed on a group of 80 patients. 40 of the patients were diagnosed with Breast Cancer after neoadjuvant polychemotherapy and 40 patients were diagnosed with Cancer of the reproductive organs in women (cervix, uterus, adnexa) associated with chemotherapy. The analysis of the results of the clinical trial took place in comparison with the data obtained prior to the beginning of the treatment. The clinical study was performed in accordance with the legislation of the Republic of Moldova and taking into account the principles of international law and the Declaration of Helsinki.

In the group of patients with Breast Cancer, who took balm for 30 days, the following was recorded: decrease in the number of patients with asthenovegetative syndrome from 50 to 5%, decrease in the number of patients with dyspeptic syndrome from 25 to 5%, of those with pain syndrome - from 15 to 5%, of those with hemorrhagic syndrome from 10 to 0%, while in the control group no changes occurred. In the group of patients with cancer of the reproductive organs there was a decrease in the number of patients with asthenovegetative syndrome from 55 to 20%, the decrease in the number of patients with dyspeptic syndrome from 25 to 5%, of those with pain syndrome - from 15 to 5%, of those with hemorrhagic syndrome from 5 to 0%, while in the control group no changes occurred.

Positive changes in the immunological and biochemical parameters were also recorded in

the experimental groups: Increase in the number of T helpers CD3 + CD4 +, increased value of immuno-regulatory index (CD3 + CD4 + / CD3 + CD8 +), decreased level of tumor necrosis factor (TNF), improved leukocyte formula, decreased level of ALT, AST, GTP, bilirubin, urea and creatinine.

GENERAL CONCLUSIONS

The aspects discovered during the accomplishment of the doctoral thesis "Technologies for obtaining curative and preventive products from raw materials of indigenous vegetal origin" can be expressed by the following general conclusions:

1. The total antioxidant capacity of the extracts from plant and cyanobacterial matter is a useful criterion for controlling the technological process of production and the efficiency of new curative-preventive balms.
2. Hydro-ethanol extracts from the Liquorice root (*Glycyrrhiza glabra L.*), Sweet Flag rhizomes (*Acorus calamus L.*), the aerian part of perforate St John's-wort (*Hypericum perforatum L.*), the aerian part of Oregano (*Oríganum vulgáre L.*), Peppermint leaves (*Mentha piperita L.*), the aerian part of Yarrow (*Achillea millefolium L.*), pine buds (*Pinus sylvestris L.*) have strong antioxidant properties, that allow them to be selected as basic components of the new curative-preventive balms [1]*.
3. The antioxidant activity of plant and cyanobacteria *Spirulina platensis* extracts depends on the concentration of the ethanol in the solution, the contact time of the solvent with the biomass and the type of extraction applied. The highest antioxidant activity is characteristic for the extracts obtained by repeated maceration of the plant biomass [1, 2, 4, 6]*.
4. The new curative-preventive balm recipes, developed within this study, ensure the obtaining of products with improved organoleptic properties [8-13]*.
5. The technologies developed for the production of new curative-preventive balms are based on a universal scheme, simple to achieve, which allows to obtain products with stable antioxidant performance, which exceeds the activity of macerate from the corresponding plant mixtures by 3.73 - 5.26 times, and that of extracts from individual plant components – by 63-190 times [2]*.
6. New curative-preventive balms have a low level of chronic and acute toxicity, determined by the alcohol content. The plant components of the balm mitigate the toxic effects of the hydro-ethanol solution of equivalent concentration.

7. New curative-preventive balms show radio-protective effect, expressed by moderate acceleration of radioactive cesium elimination from the body [3,5]*.
8. In the recommended chemotherapy treatment of Breast Cancer and Cancer of the reproductive organs in women, the additional administration of new curative-preventive balms leads to the reduction of the number of patients with asthenovegetative, dyspeptic, pain and hemorrhagic syndrome, as well as to the regulation of the ectopic immunological and biochemical parameters [9-13]*.

*(Note: * from the list of own publication)*

The obtained result that contributes to solving the **important problem** of obtaining products of natural origin with curative-preventive properties consists in the scientific basis of the use as a criterion of control of the new products efficiency, of their antioxidant capacity, the application of which led to the development of recipes and technologies for the production of 6 new conditioners with determined biological effects, intended for their use in the treatment and prophylaxis of different diseases and pathological conditions.

Personal contribution: The research design was carried out by the author under the guidance of the thesis tutor. In the materials, which reflect the content of the patents, the author has the quota according to the list of authors. The investigation of the biological effects of the new curative-preventive balms were performed within the Institute of Oncology Public Healthcare Institution under the managment of Mereuta Ion, the university professor. All other results obtained, their analysis, generalizations and conclusions belong entirely to the author.

Practical recommendations

1. It is recommended to use curative and preventive products based on natural and cyanobacterial extracts as a criterion for controlling the efficiency of their antioxidant capacity.
2. It is recommended to use Spirupotent balm in moderate sexual dysfunction, sleep and concentration disorders.
3. It is recommended to use balms of Fat-Frumos series as an adjuvant remedy in the treatment of Breast Cancer and Cancer of reproductive organs in women.

Suggestions on perspective research

1. To extand the research in order to discover the mechanisms of action of the new curative-preventive balms.
2. To continue the research on the discovery of new curative-preventive balms formulas based on indigenous raw materials.

SELECTIVE BIBLIOGRAPHY

1. ADEGBOLA, P., ADERIBIGBE, I. , HAMMED, W, OMOTAYO, T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. In: *American Journal of Cardiovascular Diseases*. 2017, nr.7(2), pp.19-32. ISSN: 2160-200X
2. BRAND-WILLIAMS, W., CUVELIER, M. E., BERSET, C. Use of a free radical method to evaluate antioxidant activity. In: *Lebensmittel-Wissenschaft und Tehnologies*. 1995, nr 28, pp. 25-30. *ISSN*, 1096-1127
3. CEPOI, L. et al. Antioxidative activity of ethanol extracts from *Spirulina platensis* and *Nostoc linckia* measured by various methods. In: *Analele Universităţii din Oradea, Fascicula Biologie.* 2009, nr XVI/2, pp. 43-48. ISSN, 1224-5119
4. DAVID, B., WOLFENDER, J.-L., DIAS, D. A. The pharmaceutical industry and natural products: historical status and new trends. In: *Phytochemistry Reviews*. 2014, nr 14(2), pp.299–315. *ISSN*, 1568-7767
5. DJORDJEVIC, S. M. From Medicinal Plant Raw Material to Herbal Remedies. In: EL-SHEMY, H. *Aromatic and Medicinal Plants - Back to Nature*. IntechOpen, 2017, p. 269-288. *ISBN* 978-953-51-2977-6
6. DORDEVIC, S., DICKOV, A., PAVKOV, S., TADIĆ, V., ARSIĆ, I., ŽUGIĆ, A. Manufacturing process of high quality phytopreparation on example of herbal sedative. In: *Medicinski Pregled*. 2013, nr 66(3–4), pp.170–176. ISSN: 0025-8105
7. FARZANEH, V., CARVALHO, I. S. A review of the health benefit potentials of herbal plant infusions and their mechanism of actions. In: *Industrial Crops and Products,* 2015, nr 65,pp. 247–258. *ISSN* 0926-6690
8. GAO, C., SHEN, J. Metabolic Factors and Adult Neurogenesis: Impacts of Chinese Herbal Medicine on Brain Repair in Neurological Diseases. In:*ZENG, B-Y., ZHAO,K. Neurobiology of Chinese Herb Medicine*, India: Academic Press, 2017, pp.117–147. ISBN: 9780128117798
9. KARGOZAR, R., AZIZI, H., SALARI, R. A review of effective herbal medicines in controlling menopausal symptoms. In: *Electronic Physician.* 2017, nr. 9(11), pp. 5826–5833. ISSN: 2008-5842
10. KIM, K.-I., JUN, J. H., BAEK, H., KIM, J.-H., LEE, B.-J., & JUNG, H.-J. Oral administration of herbal medicines for radiation pneumonitis in lung cancer patients: A systematic review and meta-analysis. In*: PLOS ONE*, 2018, nr.13(5), pp. e0198015. ISSN · 1932-6203
11. LIMA, F. A. V., JOVENTINO, I. P., JOVENTINO, F. P., DE ALMEIDA, A. C., et al. Neuroprotective Activities of Spirulina platensis in the 6-OHDA Model of Parkinson's Disease Are Related to Its Anti-Inflammatory Effects. In: *Neurochemical Research.* 2017, nr 42(12), pp. 3390–3400. *ISSN*: 0364-3190

12. MA, X.H., ZHENG, C.J., HAN, L.Y., XIE, B., JIA, J., CAO, Z.W., CHEN, Y.Z. Synergistic therapeutic actions of herbal ingredients and their mechanisms from molecular interaction and network perspectives. In: *Drug Discovery Today*. 2009, nr 14, pp.579–588. *ISSN*: 1359-6446
13. NIRUMAND, M., HAJIALYANI, M., RAHIMI, R., FARZAEI, M., et al. Dietary Plants for the Prevention and Management of Kidney Stones: Preclinical and Clinical Evidence and Molecular Mechanisms. In: *International Journal of Molecular Sciences,* 2018, nr.19(3),pp. 765. ISSN · 1422-0067
14. OHNISHI, S., TAKEDA, H. Herbal medicines for the treatment of cancer chemotherapy-induced side effects. In*: Frontiers in Pharmacology*, 2015, nr. 6.(14), ISSN: 1663-9812
15. OVANDO, C. A., CARVALHO, J. C. DE VINÍCIUS DE MELO PEREIRA, G., JACQUES, P., SOCCOL, V. T., SOCCOL, C. R. Functional properties and health benefits of bioactive peptides derived from Spirulina: A review. In: *Food Reviews International.* 2016, nr 34(1), pp. 34–51. ISSN: 8755-9129
16. RE, R., PELLEGRINI N., PROTEGGENTE, A., PANNALA, A, YANG, M., RICE-EVANS C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. In: *Free Radical Biology & Medicine*. 1999, nr 10, pp. 1231-1237. ISSN: 0891-5849
17. SAAD, B., ZAID, H., SHANAK, S., KADAN, S. Antidiabetic Medicinal Plants. In: *SAAD, B., ZAID, H., SHANAK, S., KADAN, S. Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals*. Cham: Springer, 2017, pp.59-93. *ISBN* 978-3-319-54102-0
18. SUCHER, N. J., CARLES, M. C. A pharmacological basis of herbal medicines for epilepsy. In: *Epilepsy and Behavior*, 2015. nr.52, pp.308–318. ISSN 1525-5050
19. THANAN, R., OIKAWA, S., HIRAKU, Y., OHNISHI, S., et al. Oxidative Stress and Its Significant Roles in Neurodegenerative Diseases and Cancer. In: *International Journal of Molecular Sciences.* 2015, nr 16(1), pp.193–217. ISSN · 1422-0067
20. VEERESHAM, C. Natural products derived from plants as a source of drugs. In: *Journal of Advanced Pharmaceutical Technology & Research*. 2012; nr 3(4), pp.200–201. ISSN: 2231-4040
21. WAGNER, H. Synergy research: approaching a new generation of phytopharmaceuticals. In: *Fitoterapia.* 2011, nr 82, pp.34–37. ISSN*:* 0367-326X
22. WANG, F. The roles of preventive and curative health care in economic development. In: *PLOS ONE*, 2018, nr.13(11), pp. e0206808. ISSN · 1932-6203
23. XIU, L.-J., SUN, D.-Z., JIAO, J.-P., YAN, B., et al. Anticancer effects of traditional Chinese herbs with phlegm-eliminating properties – An overview. In: *Journal of Ethnopharmacology.* 2015, nr. 172, pp. 155–161. ISSN 0378-8741
24. YAKUBU, M. T., SUNMONU, T. O., LEWU, F. B., ASHAFA, A. O. T., et al. Medicinal Plants Used in the Management of Diabetes Mellitus 2015. *In: Evidence-Based Complementary and Alternative Medicine,* 2015, nr.2015, pp.467196. ISSN: 1741-427X

25. YAO, H., QIAO, Y.-J., ZHAO, Y.-L., TAO, X.-F., et al. Herbal medicines and nonalcoholic fatty liver disease. In: *World Journal of Gastroenterology*. 2016, nr. 22(30),pp. 6890. ISSN 1007-9327..

OWN PUBLICATIONS ON THE THESIS

1. **CARAUS, V**. Extracts with antioxidant properties of plant raw materials. In: *Bulletin of the Academy of Sciences of Moldova. Life sciences*. 2019, no. 3(339), pp.124-129. ISSN: 1857-064X (in romanean)
2. RUDIC, V., **CARAUS, V.** Technology for the production of Spirupotent aphrodisiac balm. In: *Bulletin of the Academy of Sciences of Moldova. Life sciences*. 2019, no. 3(339), pp.129-135. ISSN: 1857-064X (in romanean)
3. **CARAUS, V.,** MEREUTA, I., SOFRONI, D. The curative-preventive balm "Fat Frumos" in prevention of cancer. In: *Scientific-practical journal Info-Med*, 2016, 2(28-2), p.231-234. ISSN: 1810-3936(in romanean)
4. **CARAUS, V.** Extracts of Spirulina platensis for use in the composition of curative and preventive products. In: *The materials of the scientific conference of the PhD students "Contemporary trends of the development of science: visions of young researchers"*, 8th edition, Vol. I, June 10, 2019, Chisinau, Republic of Moldova, p.67-72. ISBN 978-9975-108-66-9.
5. **CARAUS, V.** Radioprotective properties of "Fat Frumos" balm. In: *The materials of the scientific conference of the PhD students "Contemporary trends of the development of science: visions of young researchers"*, 7th edition, Vol. I, June 10, 2018, Chisinau, Republic of Moldova, p.142-146. ISBN 978-9975-108-44-7.
6. **CARAUS, V.**, RUDIC, V. Methods for obtaining spirulina medication with high antiradical properties. In: *International Scientific Symposium (5th edition) "Advanced biotechnologies - achievements and perspectives"*. October 21-22, 2019, Chisinau, Republic of Moldova, p.29. ISBN 978-9975-56-695-7.
7. **CARAUS, V.** Natural product with aphrodisiac–like effect containing spirulina extract. In: *The International Scientific Conference on Microbial Biotechnology, 4rd edition*, October 11-12, 2018, Chisinau, Republic of Moldova, p. 46. 978-9975-3178-8-7.
8. **CARAUS, V**; RUDIC, V. *Balm with aphrodisiac effect***.** Patent MD 1216 C12G 3/06. Caraus V., Rudic V. No. s 2017 0050. Date of filing: 12.04.2017. Published: 31.12.2017. in BOPI 2017, no.12., pp.49-50
9. MEREUTA, I., MD; **CARAUS, V.,** MD; MORARU, A., MD; CICALCHIN Serghei, *Balm*. Patent MD 1368 C12G 3/06. IFS. No. s 2019 0010. Date of filing: 12.02.2019. Published in BOPI, 2019, no.9, pp.47-48.

10. MEREUTA, I., MD; **CARAUS, V.,** MD; MORARU, A., MD; CICALCHIN Serghei, *Balm*. Patent MD 1369 C12G 3/06. IFS. No. s 2019 0011. Date of filing: 12.02.2019. Published in BOPI, 2019, no. 9, pp.48-49.
11. MEREUTA, I., MD; **CARAUS, V.,** MD; MORARU, A., MD; CICALCHIN Serghei, *Balm*. Patent MD 1370 C12G 3/06. IFS. No. s 2019 0012. Date of filing: 12.02.2019. Published in BOPI, 2019, no.9, pp.49-50.
12. MEREUTA, I., MD; **CARAUS, V.,** MD; MORARU, A., MD; CICALCHIN Serghei, *Balm.* Patent MD 1377 C12G 3/06. IFS. No. s 2019 0013. Date of filing: 12.02.2019. Published in BOPI, 2019, no.10, pp.53-54.
13. MEREUTA, I., MD; **CARAUS, V.,** MD; MORARU, A., MD; CICALCHIN Serghei, *Balm*. Patent MD 1378 C12G 3/06. IFS. No. s 2019 0014. Date of filing: 12.02.2019. Published in BOPI, 2019, no.10, pp.54-55.

ANNOTATION

Caraus Vladimir. "Technologies for obtaining curative and preventive products from raw materials of indigenous vegetal origin", PhD thesis in biological sciences, Chisinau, 2020

This thesis consists of an introduction, 5 chapters, general conclusions and recommendations, literature reference of 215 titles, 6 appendices, 136 basic text pages, 29 figures, 28 tables. The results are presented in 13 publications.

Keywords: curative-preventive product, balm, hydro-ethanolic extract, macerate, antioxidant activity, biological activity, radio protective effect, aphrodisiac.

Purpose: development of new technologies for obtaining products with curative-preventive properties - balms - based on the raw materials of indigenous vegetal origin and highlighting their biological effects.

Objectives: To develop efficient procedures for extracting the active components from plant and cyanobacterial biomass using environmentally friendly technologies; Development of formulas for new curative and preventive products based on plant extracts and cyanobacterial biomass; Development of manufacturing technologies for new curative and preventive products; Revealing biological effects of new curative and preventive products.

Know-how and originality of the scientific results: The efficiency of using the values of the antioxidant activity as a criterion for evaluating the curative-preventive performances of the products based on plant raw materials has been demonstrated. The cumulative/synergistic antioxidant effect of the new curative-preventive formulas, obtained from the plant extracts and spirulina and the pronounced biological effects of the new products have been shown. 6 new formulas and technologies for obtaining curative and preventive products were developed. The know-how and originality of the obtained results is supported by 6 patents for new curative and preventive products, as well as four awards at international inventions salons offered for these innovations.

Obtained result that contributes to solving the important problem of obtaining products with curative - preventive properties, consists in the scientific basis of the use as a criterion of control of the efficiency of new products of their antioxidant capacity, which application has led to the development of formulas and technologies for the production of 6 new balms with determined biological effects, intended for their use in the treatment and prevention of various diseases and pathological conditions.

Theoretical significance: New data have been accumulated that contribute to supplementing the knowledge about the antioxidant activity of the various extracts from plant biomass and about the synergistic effects in the multicomponent natural medicines, obtained from several plant sources. The utility of applying the criterion of antioxidant activity in the process of development of curative - preventive products based on plant materials was theoretically substantiated.

Applicative value: The formulas and technologies of industrial production were developed for 6 new curative and preventive products based on plant extracts and spirulina with pronounced biological effects, without toxicity and adverse effects.

Implementation of the results: Technologies for the production of new balms have been implemented at the company MAURT where experimental parties of the new balms were produced. The results obtained were also implemented in the laboratory Food and sanocreatological digestion of the Institute of Physiology and Sanocreatology as elements of the nutrition in the development of the new food systems.

TECHNOLOGIES FOR OBTAINING CURATIVE AND PREVENTIVE PRODUCTS FROM RAW MATERIALS OF INDIGENOUS VEGETAL ORIGIN

167.01. – BIOTECHNOLOGY AND BIONANOTECHNOLOGY

Summary of the doctoral thesis in biology

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