дотелиальных клеток, стимулирует их апоптоз, влияет на активность моноцитов, нейтрофилов, макрофагов, способствуя таким образом воспалительным и некротическим процессам в злокачественных опухолях. Одним из перспективных направлений таргетной терапии онкозаболеваний является использование антиангиогенных, прокоагулятивних и проапоптичних лекарственных средств, что послужило основой для выбора в качестве объекта исследований противоопухолевого цитокина EMAP II. В Институте молекулярной биологии и генетики НАН Украины разработана биотехнология бактериальной экспрессии рекомбинантного EMAP II в клетках E.coli BL21 (DE3) и выделения высокоочищенных препаратов цитокина в препаративных количествах. Для повышения стабильности и снижения агрегации рекомбинантного EMAP II разработаны научно-методические основы создания и получены нанокомпозитные комплексы цитокина EMAP II с биосовместимыми полимерами циклодекстринами и декстраном 70. В данной экспериментальной работе исследовано влияние нанокомпозитного комплекса EMAP II с декстраном-70 на организм животных с целью установления безопасности его применения. В качестве объекта исследований были использованы мыши линии BALB/C. Экспериментальные исследования показали, что при остром и хроническом введении препарата животным в дозах 300–10 000 мкг/кг не наблюдается общетоксического действия нанокомпозитного комплекса на организм мышей. Полученные данные открывают перспективу дальнейшего исследования противоопухолевых свойств нанокомпозитнигo комплекса EMAP II с декстраном-70 с целью возможного дальнейшего внедрения в фармакологическое практику.

Ключевые слова: нанокомпозитный комплекс Эмар ИИ, острая токсичность, хроническая токсичность.

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INVESTIGATION OF INFLUENCE OF EMAP II CITOKIN NANOCOMPOSITE COMPLEX WITH DEXTRAN 70 THE BALB/C LINES MUSCLE ORGANISM

The cytokine EMAP II is endothelial and monocytic-activating polypeptide II, the precursor of which is the component of the high-molecular complex aminoacyl-tRNA synthetase of the higher eukaryotes of the protein p43, is capable of modulating the properties of endothelial cells, monocytes and leukocytes. In low concentrations the cytokine stimulates and in high concentrations it suppresses the migration of endothelial cells, stimulates their apoptosis, affects the activity of monocytes, neutrophils, macrophages, thus contributing to inflammatory and necrotic processes in malignant tumors. One of the promising directions of targeted therapy of oncological diseases is the use of antiangiogenic, prokoagulative and proapoptic drugs, which became the basis for the selection of an antitumor cytokine EMAP II as an object of research. In the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, the biotechnology of bacterial expression of recombinant EMAP II in E.coli BL21 (DE3) cells and isolation of highly purified cytokine preparations in preparative amounts have been developed. In order to increase the stability and reduce the aggregation of recombinant EMAP II, scientific and methodological foundations were created and nanocomposite complexes of the cytokine EMAR II with biocompatible polymers with cyclodextrin and dextran 70 were obtained. In this experimental work, the effects of the nanocomposite complex EMAR II and dextran-70 on the animal organism were investigated for the purpose of establishing safety of its use. BALB / C mice were used as an object of research. Experimental studies have shown that acute and chronic administration of the drug to animals at doses of 300 – 10 000 μg / kg does not show the general toxic effects of the nanocomposite complex on the organism of mice. The obtained data open the prospect of further investigation of antitumor properties of the nanocomposite complex EMAR II with dextran-70 with the aim of possible further introduction into pharmacological practice.

Key words: nanocomposite complex EMAP II, acute toxicity, chronic toxicity.

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DETERMINATION OF XANTONES IN PLANTS AND THE NUTRIENT MEDIUM UNDER *IN VITRO* CULTIVATION CONDITIONS

In recent years, xanthones have received considerable attention from scientists due to their biological activity: anti-carcinogenic, antiviral, antibacterial, antioxidant, anti-inflammatory and other properties.Therefore they are useful for prevention and treatment of different diseases:cancer, Alzheimer's and Parkinson's disease, cardiovascular disorders, diabetes, etc. Extracts of different species of plants containing xanthones are components of chemotherapeutic and other medical drugs. In order to find the most sensitive and environmentally safe method of quantitative determination of xanthones in the plant material and the nutrient medium, known methods were tested and selected for the prototype Vyisochina G. I. et al., 2011 method, which uses ethanol as an extractor. As the plant material we used plants of different species that were grown under in vitro cultivation conditions on the agarized nutrient medium. This agarized nutrient medium was also used for the xanthone content analysis. Based on the performed research, modifications of the method for determining the content of xanthones were adapted to the in vitro conditions, which detail the specificity of extraction and quantitative calculation of the xanthone content in plant explants. Our own method of determination of these compounds in the agarized nutrient medium was developed as well. The method, that we proposed, will significantly speed up the process of xanthone detecting and will also increase their yield in biotechnological processes for obtaining the pharmacologically valuable secondary metabolites of phenolic nature.

Key words: xanthones, chromatography, spectrophotometry, in vitro, plants, nutrient medium.

Introduction. Xanthones – heterocyclic polyphenolic compounds, which are particularly valuable secondary metabolites. Vascular plants produce 79 % of all known xanthones of natural origin, non-lichenized fungi – 16 %, lichens – the remaining 5 % [4, 9].In recent years, these polyphenolic compounds have received considerable attention from scientists due to their biological activity, namely: anti-carcinogenic, antiviral, antibacterial, antioxidant, anti-inflammatory and other properties. Therefore they are used to prevent and treat cancer, Alzheimer's and Parkinson's disease, cardiovascular disorders, diabetes, etc. [1, 10]. Extracts of different species of plants containing xanthones are components of chemotherapeutic and other medical drugs.These compounds perform a protective function in plants: provide resistance to stress and pathogens, participate in the allelopathic interactions, as well as in the processes of general development of the plant organism [3].

The massive collection of plants as a medicinal material, which is the source of xanthones, destructively affects the species composition of natural phytocoenoses. Thus, the species of plants as sources of xanthones from the families *Gentianaceae*, *Hypericaceae* and others already have a rare and endangered status [2, 6, 7]. Consequently, it is important to search for alternative sources of xanthones, which

can be, for example, high-yielding plant tissues cultures *in vitro*. In our opinion, an additional source of xanthones may also be the nutrient medium on which *in vitro*-plants were cultivated, whose roots are known to produce secondary metabolites, including a wide range of phenolic compounds.

Several methods for the extraction of xanthones are proposed in the scientific works, but most of them are labor-intensive, have low selectivity or practical yield (a large amount of materials is needed) and, moreover, require the use of toxic organic solvents (acetone, hexane, chloroform, methanol) [8]. Such methods are ineffective in the researching of the content of plant xanthones from *in vitro* tissue cultures and the nutrient medium.

Therefore, the aim of our study was to select the most sensitive and environmentally safe method for qualitative determination of xanthones in plant material, as well as to develop a method for the extraction of these compounds from an agarized nutrient medium as an additional source of pharmacologically valuable secondary metabolites. The main task in developing the modification of the method was to minimize the amount of material needed for the effective detection of xanthones.

Materials and methods.

Chemicals. The following reagents were used in the experiment:

1. For growing plants under *in vitro* conditions: Murazige-Skuga nutrient medium (mix of macro- and microsoles) (Sigma, Germany), agar (Spain), mesoinozit (China), thiamine (LLC UKRCHIMEXPO, Ukraine), pyridoxine (LLC UKRCHIMEXPO, Ukraine), nicotinic acid (LLC "UKRCHIMEXPO", Ukraine), ascorbic acid (LLC "UKRCHIMEXPO", Ukraine), Fe_2SO_4 (LLC "UKRCHIMEXPO", Ukraine), Na-EDTA (LLC "UKRCHIMEXPO", Ukraine), kinetin (Sigma, Germany), indolyl acetic acid (Sigma, Germany), casein hydrolyzate (Sigma, Germany), yeast extract (LLC "UKRCHIMEXPO", Ukraine), sucrose (LLC "UKRCHIMEXPO", Ukraine).

2. For extraction of xanthones, chromatography and spectrophotometryanalysis: 70 % ethanol (v/v) (DKP Pharmaceutical Factory LLC, Ukraine), 40 % acetic acid (v/v) (LLC "UKRCHIMEXPO", Ukraine).

Plant material. For approbation of known methods for the xanthone determination in plant material, the roots of plants of the genus *Iris* L. were used.

For developing our own modifications of the method for the xanthone determination in *in vitro*-plants we used plants of the genus *Phalaenopsis sp.* [18] and *Acoruscalamus* L. (various variants of explants originating from different localities) [17]. The investigated plants were grown under *in vitro* conditions for 3-5 month sat a temperature of 24 °C, with a 16-hour photo period on the modified agarized Murassigu-Skooganutrient medium. *In vitro* cultivation of plants was performed in the research laboratory of "Introduced and natural phytodiversity" of O.V. Fomin Botanical Garden named of Taras Shevchenko National University of Kyiv.

Approbation of different extraction methods and quantitative determination of xanthones were carried out in the research laboratory "Physiological bases of plant productivity" of the Department of Plant Biology at the Educational and Scientific Centre "Institute of Biology and Medicine" of the Taras Shevchenko National University of Kyiv.

The agarized nutrient medium on which the plants under study were grown were also use das a material for extraction and quantitative determination of xanthonecontent.

Extraction and quantitative determination of xanthone content. In order to select the most sensitive method for the xanthone determination in plant material, we have already tested the methods known in the scientific literature (Table 1).

Table 1. **Extraction methods of xanthones from the plant material**

№	Weight of plant material, g	Volume of extractives, ml	Composition of the extraction solution	General time of extraction	References
1	1 (dry material)	30	methanol	2 hours	Krivut B. A. et al., 1976 [14]
2	1500 (dry material)	plant mat-l / ethanol = 1/12	1) 70 % ethanol (v/v); 2) chloroform (200 ml); 3) ethylacetate(300 ml)	360 hours (72 hours per cycles)	Mihaylova T. M.et al., 2005 [15]
3	dry material	–	1) 96 % ethanol (v/v); 2) 40 % ethanol (v/v); 3)hexane 4) chloroform	4 cycles (timeis not specified)	Munhzhargal N et al., 2008 [16]
4	1700 (dry material)	–	1) ethanol; 2) chloroform 3) butanol	6 cycles (timeis not specified)	Tozhiboev M. M. et al., 2010 [20]
5	0,5 (dry material)	50	60 % ethanol (v/v);	2 hours (1 hour for the 1-st cycle; for 0,5 hoursfor 2 subsequent cycles)	Vyisochina G. I. et al., 2011 [12].
6	1	plant mat-l / ethanol= 1/ 10	1) 96 % ethanol (v/v); 2) 40 % ethanol (v/v); 3) 96 % ethanol (v/v) + chloroform (5 ml)	4 cycles (time is not specified)	Tarasova N. S et al, 2012 [19].
7	0,2 (dry material)	50	80 % methanol (v/v)	1 hour	Liu X. et al., 2014[5].

Quantitative analysis of xanthone content was carried out using paper chromatography and spectrophotometrically (SHIMADZU UV-1800, Japan) at λ = 200–400 nm.

Statistical analysis. Statistical processing of the results was carried out using common methods using the standard program Microsoft Office Excel 2007. The biological repetition of the experiments was 3 times, the analytical repetition – 9 times. In preparation of the method, the average values of the indices were analyzed, the differences between which were considered significant at $P \leq 0.05$ according to the Student's criterion [13].

Results and discussion. On the basis of the elaborated publications [5, 12, 14–16, 19, 20], we have developed our own modifications of methods for the xanthonedetermination in plants grown under *in vitro* conditions. Since in literary sources we did not find methods for xanthone extraction from agarized nutrient medium, we have developed our own method of isolating these compounds and determining their content in the agarized nutrient medium.

Extraction of xanthones from plant material. The methods of extraction and determination of the xanthone con-

tent (see Table 1) presented in the literature are different in terms of the amount of dry plant material (from 0,2 g (№ 7) to 1,7 kg (№ 4)), by the composition of the extraction solution (methanol, ethanol, hexane, chloroform (№ 3), ethanol, chloroform, butanol (№ 4), ethanol, chloroform (№ 6)), as well as the duration extraction (from 1 hour (№ 7) to 360 hours (№ 2)). The presence of toxic solvents in solutions for extraction does not allow the use of extracts obtained without further purification.

Our analysis of the data of xanthone extraction methods has shown that method №5 is the most environmentally friendly and requires a small amount of materials. Therefore, this method became the basis for developing a modified version:

– weigh 0,1–0,3 g of raw plant material gomogenise in a porcelain mortar with a small amount of 70 % ethanol (v/v);

– add 70 % ethanol (v/v) and transfer quantitatively into heat resistant conical flasks (volume 100 ml) with reflux condensers so that the total volume of the extract does not exceed 4–5 ml;

– extract in a boiling water bath for 3 hours;

– the cooled extract filtere through a Shota-filter in a heat-resistant flask; add a 70 % ethanol (v/v) portionwise (0,3–0,5 ml) to the Shota-filter several times until the solution slips from the filter, does not become colorless; combine filtrate;

– heat-resistant flasks with the resulting total filtrate place on a water bath and drop the contents to dryness (if necessary, cooled flasks with a dry residue hermetically sealed with rubber stoppers and left for 24 hours at a temperature of 3–7 °C);

– add 0,5–1 ml of 70 % ethanol (v/v) to the dry residue (for better dissolution of the remainder of the flask can be heated to 60° C);

– to carry out paper chromatography in the ascending method, take a chromatographic paper 8 x 11 cm, marking the starting line at a distance of 1 cm from the lower edge; obtain the extract step by step on the marked line (at the same time at each stage it should be completely dried); add thrice (0,1-–0,2 ml) 70 % ethanol (v/v) in small portions to wash the remainder of the extract from the flask, apply it to the starting line;

– upstream chromatography in a small chromatography chamber using 40 % acetic acid (v/v) as a mobile phase (the time required for chromatography is 15–20 minutes under normal conditions);

– dry the chromatograms, place under the UV-irradiation, bright orange spots of xanthones circle with a pencil;

– cut blotted spots, grind, place in test tubes and add 4 ml of 60 % ethanol (v/v), seal the tubes with rubber stoppers and leave them for 6 hours;

– in the case of saturated bright orange color of the obtained eluates (which indicates the high concentration of xanthones in the experimental plant material), they must be diluted with 60 % ethanol (v/v),and then multiplicity of this dilution should be taken into account during calculations;

– the eluates obtained should be used for spectrophotometric analysis (the range of wavelength to be used for the analysis is λ = 200–400 nm);

– to calculate the xanthone content in 1 g of raw plant material, use the formula:

$$C = \frac{D * z * 10}{P * 1 * M * E_{1cm}^{1\%}}, \text{ where}$$

C – content of xanthone in the sample,%; D – optical density of the investigated solution; M – molecular weight of xanthone compound; l – path length of sample (cuvette thickness), cm; $E_{1cm}^{1\%}$ – specific absorption coefficient of xanthone compound; P – weight of plant material, g; z – multiplicity of dilution of the eluate used for spectrophotometric analysis.

Extraction of xanthones from the agarized nutrient medium. In the vast majority of cases, extraction of secondary metabolites is carried out from a liquid nutrient medium in the cultivation of suspension cultures [20]. For the effective use of *in vitro*-cultures of plants grown on an agarized medium, along with the modification of the xanthone extraction from plant material, a method for extracting xanthones from agarized nutrient medium was developed. In the process of developing the method, the weight of the initial weight gain of the agarized nutrient medium was 0,1 g. However, we failed to establish the statistically significant values of the studied parameters. With an increase in cravings of the nutrient medium up to 0,5 g, the determination of the xanthone content was successful.

Thus, the following sequence of analysis of agarized nutrient medium has been developed:

– homogenize the nutrient medium (0,5 g) and place it in a 100 ml flask;

– add 3 ml of 70 % ethanol (v/v) to the homogenate, mix thoroughly, close with rubber stoppers and withstand under the temperature of 5 °C for 24 hours;

– transfer the samples quantitatively to centrifuge tubes and centrifuge for 10 min. at 2000 rpm;

– take the supernatant carefully into the test tubes;

– add 1 ml of 70 % ethanol (v/v) to the precipitate remaining in the centrifuge tube, mix and centrifuge for 10 min.;

– take the supernatant obtained and combine with the previous extract (repeat the procedure 2 more times) and use for chromatographic analysis;

– the quantitative determination of xanthones is carried out using the same method as in the plant material.

With the help of developed methods it was found that the xanthone content in *in vitro*-plants remained stable or increased during cultivation, and in the nutrient medium – decreased. Consequently, for the biosynthesis the maximum amount of xanthonesin the *in vitro*-plants, it is necessary to regulate the plant cultivation conditions, in particular the duration of cultivation [17, 18].

In conclusion, an ecologically safe and effective for a small amount of experimental material modification to the method for xanthone determination in plant material was selected and a method for their extraction from the agarized nutrient medium was developed. The method, that we have proposed, will significantly speed up the process of detecting xanthones and also increase their yield in biotechnological processes for obtaining a pharmacologically valuable substance.

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ВИЗНАЧЕННЯ КСАНТОНІВ У РОСЛИНАХ ТА ЖИВИЛЬНОМУ СЕРЕДОВИЩІ ЗА УМОВ КУЛЬТИВУВАННЯ *IN VITRO*

В останні роки вторинні метаболіти, зокрема ксантони, отримали значну увагу науковців завдяки своїй біологічній активності: антиканцерогенним, противірусним, антибактеріальним, антиоксидантним, протизапальним та іншим властивостям. Тому вони використовуються для профілактики та лікування різних захворювань: раку, хвороб Альцгеймера та Паркінсона, порушень серцево-судинної системи, діабету тощо. Екстракти різних видів рослин, що містять ксантони, є компонентами хіміотерапевтичних та інших лікарських засобів. Щоб підібрати найбільш чутливий і екологічно безпечний метод якісного та кількісного визначення ксантонів у рослинному матеріалі та агаризованому живильному середовищі, було апробовано відомі методи та відібрано як прототип метод Vyisochina G. I. Et al., 2011, який застосовує етиловий спирт як екстрактор. Як рослинний матеріал ми використовували рослини різних видів, які вирощували в умовах культивування in vitro на агаризованому живильному середовищі. Це живильне середовище також було використане для аналізу вмісту ксантонів. На основі здійснених досліджень модифікації методу визначення вмісту ксантонів