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Isolated Culture of St.-John's Wort Elongated (*Hypericum Elongatum* Ledeb.) as the Source of Biologically Active Compounds

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Keywords: *isolated cultures, Hypericum elongatum, secondary metabolites* .

Healthy properties of medicinal plants and their practical application are conditioned by the century-old experience when medicinal plants were the single accessible healing means. Now, in spite of successes achieved by the synthetic chemistry, medicinal preparations of plant origin occupy the significant place in the modern pharmacy. Because of the complexity of chemical composition of many biological active combinations of plant origin the made attempt of their chemical synthesis are not always successful and limited often by the low output and high cost. The synthesis in the plant themselves in many cases appears to be more effective [1]. The medicinal plants attract the great attention as sources of antibiotic preparations too. Antimicrobial properties are detected practically for all plants [2]. Antibiotics of microbial origin exert influence upon the immunobiological reactivity of macroorganism, break the formation of specific immunity [3]. Among the substances of plant origin, which have even the weak antibiotic influence, the active stimulators of immunological reactivity of organisms are found often [2]. However steady growth of industry, the population increasing with the growing consumption of the sources of raw materials and the utilization of several years' standing of wild useful plants resulted in the cutting down of natural stocks of many of them. In this connection one of the actual tasks confronting the resource studying is the search of new sources of raw materials.

For getting physiological active substances the biological methods of obtaining the raw can be used, in particular by way of the cultures of isolated tissue of plants. There are sufficient data about the synthesis by isolated cultures

the substances, which are identical to those synthesized by the whole plant [4-8].

Owing to the production of naphthadianthrons and hyperforin St.-John's wort perforated (*Hypericum perforatum*) is the wide-used medicinal plant [9]. Antibacterial activity of dianthrons with respect to the different gram-negative and gram-positive microorganisms was shown in a number of works [10-14]. Equally with the St.-John's wort perforated other species this genus - St.-John's wort elongated (*Hypericum elongatum*) also is recommended by scientific medicine: it has identical composition of the products of secondary metabolism [9].

The goal of the present paper was obtaining of isolated tissues of *H. elongatum* and study of antimicrobial activity of extracts from such cultures.

Methods and material. Flower buds of plants, collected on the parcel of Armenian flora of the Institute of Botany of NAS RA, serve as explants for introducing into isolated culture. Callus tissue was obtained according to the adopted method on the modified medium of Murashige and Skoog (MS) [16], which contained 1 mg/l kinetin, 0,5 mg/l naphthylacetic acid (NAA), 0,2 mg/l gibberellic acid (GA) and 3 % saccharose. The callus tissues were grown up subsequently both on the above mentioned medium and on the media of other composition and ratio of phytohormones using also benzylaminopurine (BAP) and indole-3-acetic acid (IAA).

For characterization of grow activity of callus cultures, the grams of crude weight, % of dry mass, growth index (GI) – ratio of callus crude weight at the end of cultivation to the initial weight, were determined.

For the anatomical investigations thin sections of callus cultures were died by the water solutions of saphranine (1%). Surplus of dye were removed by the immersing of sections into glycerin for 1-2 days.

Antibacterial activity was determined by the method of wells [17]. For the preparation of extracts one gram of dry tissue was extracted in 10 ml of 40 % ethanol for 2 hours on the magnetic rocking-chair at the temperature $4^{O}C$. Obtained suspension was centrifuged, and supernatant was used as crude extract of antibiotic. The experiments were carried out thrice-repeatedly. Gram-positive and gram-negative microorganisms were used as test-microorganism. 40% ethanol, which did not suppress the growth of microbes, was used as the control.

The determination of antioxidant activity was carried out by permanganatometric method [18].

Results and discussion. The first signs of callus formation on explants were observed at 18-20 days of cultivation on the medium with 1 mg/l kinetin, 0,5 mg/l NAA and 0,2 mg/l GA (Table 1, medium N 4). But further growth of callus on this medium was not observed. Due to it the initial callus subsequently was transferred on the nutrient medium N 1, where GA was excluded and IAA was added instead of NAA.

Table 1

Hormonal composition of the modified Murashige and Skoog medium

Medium N	Phytohormones concentration, mg/l				
	IAA	NAA	BAP	Knetin	GA
1	1.0	0.0	0.0	1.0	0.0
2	0.5	0.0	2.0	0.0	0.0
3	2.0	0.0	0.0	0.2	0.0
4	0.0	0.5	0.0	1.0	0.2
5	1.0	0.5	0.0	1.0	0.0
6	0.0	0.1	0.0	0.0	0.0

Callus obtained on this medium was grayish and had compact consistence. The rate of growing callus mass was low, but callus had high organogenic potential. Both under the light and in the dark such callus formed many short-cut shoots (Fig. 1). Other nutrient media with different content and ratio of phytohormones were tested to intensify the growth of callus tissue (Table 1). However on the all tested media the shoot organogenesis was also observed. Anatomical investigations showed that upper layers of callus consisted of small meristematic cells and contained large amount of shoot buds (Fig. 2).

Fig.1. Organogenesis of H. elongatum callus of on the nutrient medium N 1.

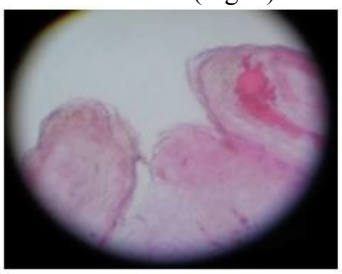

Fig. 2. Anatomical structure of shoot buds callus H. elongatum (x10).

To obtain non-differentiated callus mass the upper layer of callus with meristematic cells was removed, but low layers with parenchymatic cells were transferred on the fresh nutrient media and cultivated in the dark. As a result during 2-3 such manipulations the homogeneous non-differentiated callus tissue was obtained. As for the upper layer of callus with shoot buds it was used for the clonal micro propagation. For this aim it was transferred on the nutrient medium, which contained besides the salts and vitamins only 0.1 mg/l NAA. On this medium the shoots became longer and the roots were formed (Fig. 3). Clonal micro propagation was realized by the fragmentation of the bundle of shoots and periodic subcultivation on the fresh medium. On the nutrient medium with the equal content of auxins and cytokinins (Table 1, medium N 1) the callus was light yellow, here and there orange, it had friable consistence, and the growth of callus went on vertically. When content of cytokinin was increased (medium N 2) the callus acquired motley coloration and consisted of the orange, grey and greenish parts, it had compact consistence. When the

content of auxin in the medium was increased (medium N 3) the growth of callus mass went on the surface of the agar; callus had middle consistence and motley coloration. At the end of the cycle of growth (25-30 days) callus tissue on all media became dark, which is probably related with the accumulation of products of secondary metabolism. It is confirmed by the anatomical investigations too: callus tissue consisted of the large vacuolated cells, colored different colors – yellow, orange, red – due to the synthesis of pigments and secondary products, which were characteristic to the intact plant [19].

Fig. 3. Clonal micro propagation of H. elongatum on the medium with the 0.1 mg/l NAA.

To evaluate the growth activity of callus tissue the dynamics of accumulation of crude and dry mass, according to the days of cultivation on the all tested media, was studied. The growth of callus on the medium N 1 with the equal quantities of auxin and cytokinin gave the increased curve and reached its maximum on 25 days of cultivation – GI was equal to 9. Dry mass at this time was almost 90 % (Fig. 4). On the other media the growth was 1.5 – 2.0 times lower, and accumulation of dry substances did not differ almost from the one on the medium N 1. Such low rate of tissue growth can be explained by its high metabolic activity [20].

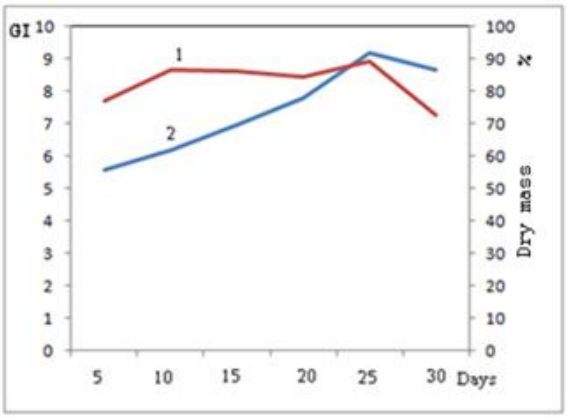

Fig. 4. Growth indices of callus tissues of *H. elongatum* on the medium with equal quantities of auxin and cytokinin 1. Dry mass. 2. GI.

As it was mentioned above, the dianthrones, synthesized by *H. perforatum*, had antimicrobial activity. As for the composition of synthesized products of the secondary metabolism the plants *H. elongatum* can be used in medicine on a level with *H. perforatum*. Therefore we studied antibacterial activity of extracts of *H. elongatum* callus tissues, obtained in our experiments. The extracts of the intact plant served as a control.

According to the obtained data the callus tissues synthesize the substances possessing antimicrobial activity against both gram-positive, including spore-forming bacteria, and gram-negative microorganisms, as well as against some pathogens (Table 2, Fig. 4). So, when in the nutrient medium there were in equal quantities cytokinins and auxins, the produced substances inhibited the growth of *Staphylococcus aureus,* similar to the action of control; the action of these substances on *Bacillus mesentericus* even surpassed the control. When in the medium the content of BAP was increased, the substances were synthesized, which inhibited the growth of *Salmonella typhimurium* and *Bacillus mesentericus* more actively, than the intact plant; on the other hand, this extract had the same effect as intact plant on *Staphylococcus citreus*. The increase of content of IAA in the medium resulted in the synthesis of substances with high inhibitory influence on the growth of *Bacillus mesentericus*: the zone of the growth absence was 5-6 times larger than in the case of control (intact plant). The substances, synthesized on this medium, in comparison with the intact plant and preceding media, inhibited also more actively the growth of *Staphylococcus citreus*.

Table 2

Inhibition of test- microorganisms under the influence of extracts from callus cultures of H.elongatum

Culture used	Test -organisms					
	E. coli M17	*St. citreus*	*St. aureus 5233*	*B. subtilis*	*B. mesent.*	*S. typh.*
	Diametrs of growth absence zone of test -microorganisms (Ømm)					
Intact plant	33	13	32	38	5	29
Callus(medium IAA/cytokinin 1:1)	19	7	28	16	10	5
Callus (medium IAA/ BAP 1:4)	24	13	14	22	15	34
Callus (medium IAA /cytokinin10:1)	19	20	16	19	29	21

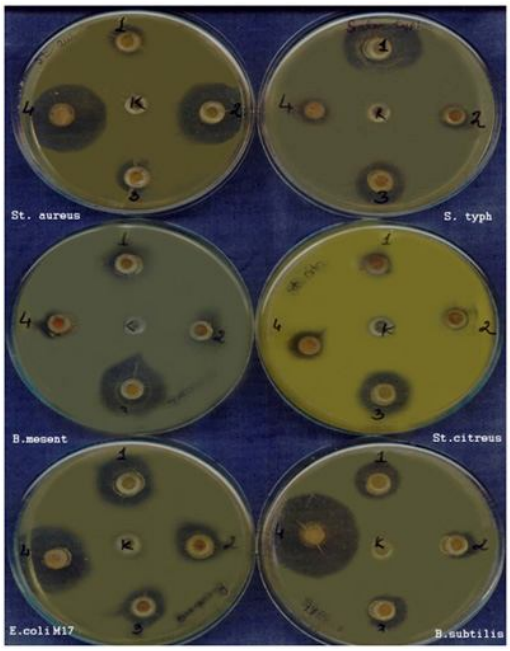

Fig. 5. Zones of growth absence around the callus extracts of H.elongatum 1 – extracts of callus grown in the nutrient medium IAA / BAP 1:4; 2 – extracts of callus grown in the nutrient medium IAA /cytokinin 1:1; 3 – extracts of callus grown on the(medium IAA / cytokinin 10:1; 4 –extracts of intact plants; 5 – control (40%- etanol).

Preliminary determination of antioxidant activity of callus tissues extracts showed its low level as compared to the intact plant.

Thus the conclusion can be done that the callus cultures of *H.elongatum* not only kept the ability to the synthesis of substances with antibiotic activity, but were able to synthesize the substances, which were not peculiar to the intact plant. At the same time the synthesis of the substances with antibacterial activity was on the hormonal control, and these substances selectively affect on the test-microorganisms.

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Isolated Culture of St.-John's Wort Elongated (*Hypericum Elongatum* Ledeb.) as the Source of Biologically Active Compounds

The isolated culture of St.-John's wort elongated (*Hypericum elongatum* Ledeb.) with high biosynthetic activity was obtained. Synthesized products of the secondary metabolism exhibit antibacterial activity against some gram-positive and gram-negative microorganisms. The influence of different hormonal substances and their various con-

centrations both on the growth of callus tissues and the synthesis of the products of secondary metabolism was studied.

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Изолированная культура зверобоя вытянутого (*Hypericum elongatum* Ledeb.) как источник биологически активных соединений

Получена изолированная культура зверобоя вытянутого (*Hypericum elongatum* Ledeb.), обладающая высокой биосинтетической активностью. Синтезируемые продукты вторичного обмена проявляют антибактериальную активность по отношению к некоторым грамположительным и грамотрицательным микроорганизмам. Изучено влияние различных гормональных соединений и их концентраций как на рост каллусных тканей, так и на синтез в них продуктов вторичнего метаболизма.

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ՀՀ ԳԱԱ թղթակից անդամ Ա. Հ. Թռչունյան

Սրոհունդ երկարավունի (*Hypericum elongatum* Ledeb.) մեկուսացված կուլտուրան որպես կենսաբանորեն ակտիվ միացությունների աղբյուր

Ստացվել է Սրոհունդ երկարավունի (*Hypericum elongatum* Ledeb.) կենսասինթետիկ բարձր ակտիվությամբ օժտված մեկուսացված կուլտուրան: Սինթեզված երկրորդային փոխանակության նյութերը հակաբակտերիական ակտիվություն են դրսևորում որոշ գրամ դրական և գրամ բացասական միկրոօրգանիզմների նկատմամբ: Ուսումնասիրվել է տարբեր հորմոնային նյութերի և դրանց տարբեր կոնցենտրացիաների ազդեցությունը ինչպես կալուսային հյուսվածքի աճի, այնպես էլ նրանցում երկրորդային նյութափոխանակության արգասիքների սինթեզի վրա:

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