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DEVELOPMENT OF PROTOTYPES OF THERAPEUTIC AND PROPHYLACTIC ANTIOXIDANT AGENTS BASED ON NATURAL BIOLOGICALLY ACTIVE COMPOUNDS OF TROPICOGENIC REPRESENTATIVES OF *ORCHIDACEAE* JUSS.

Introduction. *Plant extracts and bioactive substances (BAS) of natural origin possess a high biological activity and a low toxicity, therefore they are widely used in cosmetic, pharmaceutical, and food industries.*

Problem Statement. *Nowadays, about 50% of all medical drugs, dietary supplements, and herbal medicines are produced from natural sources, so the search for plants with a high content of BAS, the development of biotechnologies for BAS production, and the design of composites providing the most effective use of BAS are of great importance.*

Purpose. *The purpose is to study tropical orchids collection for identification of plants with a high BAS content; to develop a procedure for growing promising species in vitro; to design drug prototypes on the basis of plants extracts, nanosized silica, and gelatin biopolymer.*

Materials and Methods. *The extracts prepared from orchids leaves, silica, and gelatin have been used as auxiliary components for composites synthesis. Spectrophotometric, chromatographic, quantum-chemical, anatomo-morphological, phy-siological-biochemical, and biotechnological methods have been used in the research.*

Results. *The species of orchids, which contain a significant amount of BAS with high antioxidant properties have been identified, the main components of orchids extracts have been distinguished. Sterile cultures of selected species have been obtained, protocols for their cultivation in vitro have been developed. Two types of bioactive composites have been prepared, the interaction of extracts components with silica surface and gelatin molecules has been studied. It has been shown that the materials composed of silica and orchid extracts possess a high storage stability and a prolonged desorption of several BAS, while the materials based on extracts, silica, and gelatin ensure gradual release of all the extracts components.*

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Conclusions. *The orchids are valuable sources of natural antioxidants. The designed composites are promising in terms of producing drug formulations for prolonged release of antioxidants.*

Keywords: Orchidaceae Juss. extracts, polyphenolic compounds, nanosized silica, gelatin, and bioactive composites.

Comprehensive study of plant extracts is one of the priorities of modern research in the field of biology, as evidenced by Scopus data at the end of 2019 (194,735 research publications with the keyword "plant extracts"). The rate of use of herbal medicines and food formulations based on raw plant materials continues growing rapidly around the world [1, 2]. According to the Global Industry Forecast, in 2019, the market of plant extracts amounted to USD 23.7 billion and, by 2025, it is expected to get to USD 59.4 billion [3].

Plant extracts are a valuable source of natural antioxidants (AO), in particular, flavonoids and phenolic acids, which are widely used to inhibit or to slow down free radical / oxidative processes [4, 5]. The recent decline in medicinal plant populations and environment degradation, on the one hand, and the growing demand of the cosmetics, pharmaceutical, and food industries for efficient and inexpensive natural AOs, on the other hand, require the selection of available raw materials and the development of optimal biotechnological processes. In the context of creating technologies for obtaining efficient and cheap AOs, an important point is the initial selection of plants that are potentially promising in terms of the extraction of bioactive products. The development of technological processes for rapid biomass growth is also essential for solving the problem of obtaining BAS on an industrial scale. It is possible to increase phytomass and quantitative content of biologically active substances in it by creating special conditions for cultivation of representatives of the studied species *ex situ* and *in vitro*.

According to the data of scholarly research literature, the current range of formulations with plant extracts in the pharmaceutical market of Ukraine is insignificant and is mostly represented by liquid dosage forms with a limited list of original raw medicinal plant materials: chamomile flowers, sage leaves, yarrow herb, and marigold flowers [8]. Therefore, the search for raw plant materials rich in natural AOs and the creation of new formulations based on plant extracts in various dosage forms remains relevant.

The purpose of this research is to identify potential producers of BAS among botanical specimens of the orchid collection of the Gryshko National Botanical Garden of the NAS of Ukraine, to develop a technology for *in vitro* accumulation of their biomass, to create prototype formulations based on extracts from plants of the orchid family, nanosized silica, and gelatin.

The research has been done within the framework of the targeted comprehensive interdiscip-

Table. **Study of Extracts of Species from *Dendrobium* Sw. Collection of the NBG of the NAS of Ukraine**

Taxon	Flavonoid content, %	Number of DPPH radicals inhibited by the extract during 30 min, %	Origin of plant sample
Dendrobium aphyllum (Roxb.) C.E.C.Fisch.	1.172	16	← *ex situ*
	0.806	12	← *in vitro*
Dendrobium nobile Lindl.	1.860	28	← *ex situ*
	1.274	12	← *in vitro*
Dendrobium chrysanthum Wall. ex Lindl.	1.674	82	← *ex situ*
	1.071	12	← *in vitro*
Dendrobium phalaenopsis Fitzg.	1.274	38	← *ex situ*
	1.065	17	← *in vitro*
Dendrobium draconis Rchb.f.	1.736	61	← *ex situ*
	1.330	29	← *in vitro*
Dendrobium moniliforme (L.) Sw.	1.110	51	← *ex situ*
	1.020	32	← *in vitro*
Dendrobium linguella Rchb.f.	2.243	31	← *ex situ*
	1.589	14	← *in vitro*
Dendrobium lomatochilum Seidenf.	0.952	45	← *ex situ*
	1.172	43	← *in vitro*

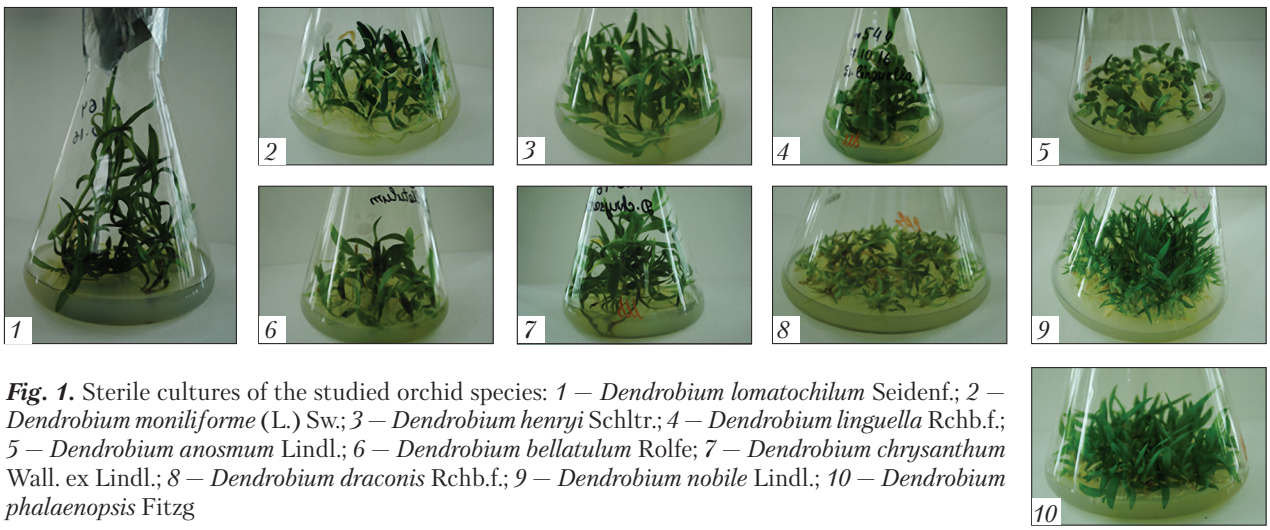


Fig. 1. Sterile cultures of the studied orchid species: *1 — Dendrobium lomatochilum* Seidenf.; *2 — Dendrobium moniliforme* (L.) Sw.; *3 — Dendrobium henryi* Schltr.; *4 — Dendrobium linguella* Rchb.f.; *5 — Dendrobium anosmum* Lindl.; *6 — Dendrobium bellatulum* Rolfe; *7 — Dendrobium chrysanthum* Wall. ex Lindl.; *8 — Dendrobium draconis* Rchb.f.; *9 — Dendrobium nobile* Lindl.; *10 — Dendrobium phalaenopsis* Fitzg

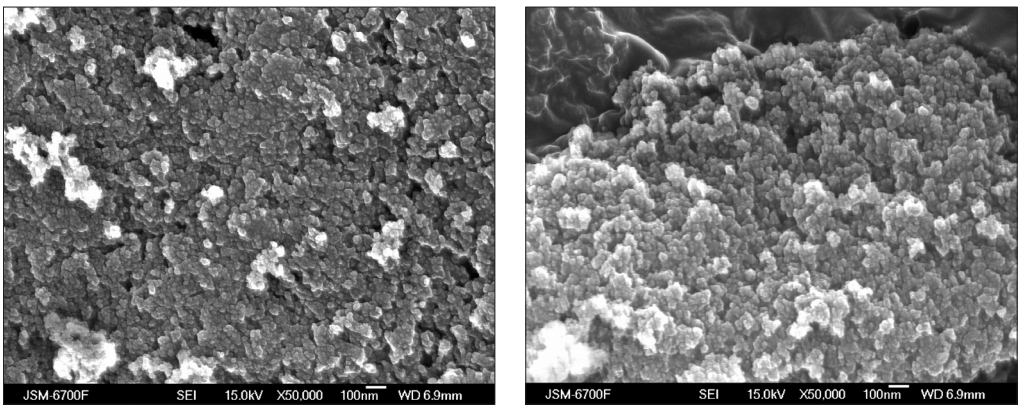
linary research program of the NAS of Ukraine *Molecular and Cellular Biotechnology for Medicine, Industry, and Agriculture* (approved by Resolution of the Presidium of the NAS of Ukraine dated 11.02.15 No. 22) and the President's grant to DSc for research 2019.

To search for valuable genotypes of plants with the highest content of biologically active substances, a collection of tropical orchids has been screened, valuable compounds have been extracted and identified, and their antioxidant properties have been described.

According to the results of screening, species whose plant extracts have shown high antioxidant properties have been identified. Particular attention is paid to 16 samples of extracts containing flavonoids. The content of flavonoids has been determined with the use of test reactions with aluminum chloride solution. The antioxidant properties of the extracts have been studied by the DPPH test [9]. The results of biochemical screening of some orchid species are presented in Table.

Having analyzed the results obtained on the quantitative characteristics of extracts of the studied species of orchids, one can note that in the case of the plants from greenhouses, the content of active substances in them, as a rule, is higher than in the case of cultivation in aseptic culture. The only exception is *D. lomatochilum*, for which extracts from the plants grown *in vitro* have a higher content of flavonoids. Four samples of the plants grown ex situ and one sample of the plants grown *in vitro* have shown a fairly high antiradical activity as they inhibit more than 50% of free radicals of DPPH. In most cases, the plants grown ex situ have higher antioxidant properties than the plants grown *in vitro*. In the case of *D. aphyllum* and *D. lomatochilum*, the extracts of plants grown *ex situ* and those of plants grown *in vitro* have almost the same activity in the reaction with DPPH.

The composition of plant extracts of the studied orchid species has been analyzed by the high performance liquid chromatography (HPLC) and the mass spectrometry (MALDI) methods. It has been found that the main classes of biologically active substances are flavonoids, hydroxycinnamic and hydroxybenzoic acids. In the extracts, flavonoids are represented mainly by flavonols and flavones. Quercetin, kaempferol, isoramnetin, apigenin, and luteolin have been found to be the most common aglycones of certain flavonoids. Derivatives of anthracene and phenanthrene, alkaloids, coumarins, furanocoumarins, and terpenoids have been detected as well. Catechin has



a *b*

Fig. 2. Original silica (a) and composite with extract of *Dendrobium nobile* Lindl. (*b*); SEM, ×100 000

been found in the extract of *D. chrysanthum* (*ex situ*). It has been established that, in general, more active substances are accumulated in the phytomass of the plants grown in greenhouses, while the content of hydroxycinnamic acids is higher in the plants grown in aseptic culture.

In parallel, a bank of axenic cultures of promising valuable species of the orchid family (Fig. 1) has been created. Based on the analysis of biological features of the plants and the specifics of their natural growth, due to the observations of the annual cycle of growth and development of generative plants in the greenhouse culture, the researchers of the NBG of the NAS of Ukraine have managed to select methods for sterilization of primary explants and to determine optimal ways for introducing each experimental species in the *in vitro* culture. The prescriptions of nutrient media for the first and subsequent stages of their reproduction in the conditions of aseptic culture have been developed. A set of measures to obtain viable seeds of several experimental species has been implemented; procedures for their further sterilization have been designed. The initial stages of growth and development of juvenile plants of experimental species *in vitro* have been described. Based on the plants used in the experiments, a bank of axenic cultures has been created. It may be used to obtain pharmaceutically valuable compounds and be involved in programs for restoration of populations of these species *in situ* or for exchange by dilectus.

Based on the plant extracts and excipients (nanosized silica and gelatin biopolymer), two types of composite materials with antioxidant properties have been developed. The interaction of the components of the extracts with the silica surface and with gelatin molecules has been studied.

According to the results of morphological and structural studies, it has been shown that the composites obtained by adsorption / deposition of the plant extracts on the surface of nanosized silica almost completely retain its initial dispersity and consist of particles with a size of 20—30 nm (Fig. 2).

With the use of the IR spectroscopy and quantum chemical methods, the formation of bonds between silanol groups of silica and OH groups of the components of the extracts has been established, the desorption of biologically active substances from the composite surface into the ethanol solution has been studied. The obtained results have proved that different phenols differ significantly from each other by interaction with silica nanoparticles and are desorbed into solution at different rates. It has found that for different groups of phenols the length of hydrogen bonds between the OH groups of molecules and

the silanol groups of silica, as well as the free adsorption energy differ significantly (0.17—0.28 nm and —56—54 kJ / mol). In this case, the presence of a phenolic ring with two adjacent OH groups in the structure of BAS molecules ensures their strong interaction with the silica surface.

The antioxidant properties of the extracts and composites based on extracts and nanosized silica stored throughout the year have been studied. Based on the identified differences in the values of antiradical activity against DPPH for the original extract and the composite based on it (Fig. 3), it has been found that the adsorption of plant extract components on the surface of silica improves their stability and extends their shelf life.

The obtained results have laid foundation for the use of nanosized silica as a carrier of BAS while developing prototype formulations.

To create formulations with prolonged action, a method for obtaining silica-gelatin materials has been proposed. It allows the introduction of biologically active substances or plant extracts during their preparation [10]. It has been shown that silica-gelatin materials are characterized by less swelling in aqueous solutions as compared with gelatin films and, accordingly, have a lower rate of desorption of active substances. To obtain therapeutic and prophylactic agents with prolonged action, the active extracts have been introduced into silica-gelatin materials in the form of thin films. In such materials, silica plays the role of a kind of crosslinking agent that forms additional bonds with gelatin molecules, slows down the swelling and dissolution of the material in the aqueous medium, and enables the controlled release of the active substance. Some active substances contained in the plant extracts may act as crosslinking agents and provide additional slowdown of swelling, facilitation of dissolution of materials, and prolongation of the antioxidant action. It has been found that high concentrations of flavonoids in silica-gelatin suspensions may affect the interaction of silica and gelatin through reducing the silica-induced effect of slowing down the swelling of films and desorption of incorporated substances. The free Gibbs energies for the interaction of the flavonoid quercetin, gelatin, and silica with each other have been calculated. The obtained data have confirmed the possibility of the effect of quercetin adsorption on the silica surface on the interaction of silica and gelatin.

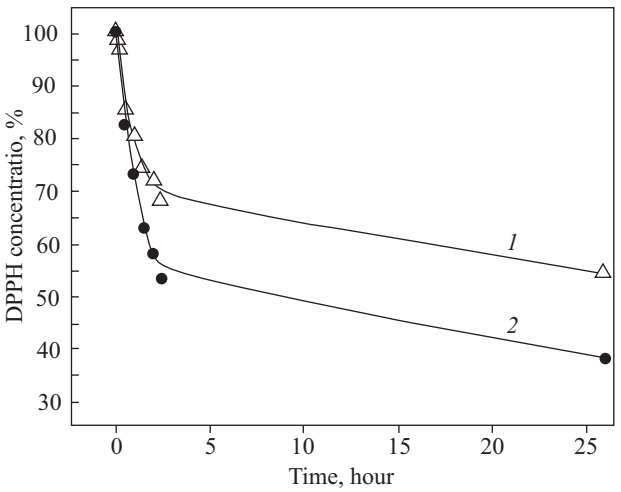


Fig. 3. Kinetic curves of inhibition of DPPH radicals in the reaction with original extract (*1*) and with composite based on it (*2*)

As a result of screening of the NBG collection of tropical orchids, some species containing a significant amount of BAS have been identified. Phenolic acids and flavonoids have been shown to be the main components of orchid extracts.

Axenic cultures of selected species have been obtained and methods for their *in vitro* cultivation have been developed. It has been found that the orchids grown *in vitro*, under certain cultivation conditions, may accumulate more BAS than the plants grown *ex situ*.

It has been shown that the presence of two adjacent hydroxyl groups in the phenolic ring of hydroxycinnamic acids or in the B-ring of flavonoids contributes to a stronger interaction of such compounds with the silica surface. The use of nanosized silica as an excipient in the creation of composites contributes to the stability of biologically active substances that are part of the extracts and extends their shelf life.

A method for obtaining silica-gelatin materials has been proposed. It allows introducing BAS, while producing the materials.

A prototype formulation with prolonged action has been created. It provides uniform release of all components of the extracts into the solution, slowdown of the flavonoid desorption, and prolonged action of antioxidant compounds.

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РОЗРОБКА ПРОТОТИПІВ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНИХ ЗАСОБІВ АНТИОКСИДАНТНОЇ ДІЇ НА ОСНОВІ ПРИРОДНИХ БІОЛОГІЧНО АКТИВНИХ СПОЛУК ТРОПІКОГЕННИХ ПРЕДСТАВНИКІВ *ORCHIDACEAE* JUSS.

Вступ. Рослинні екстракти і біоактивні сполуки (БАС) природного походження характеризуються високою біологічною активністю і низькою токсичністю, що обумовлює їх широке застосування в косметичній, фармацевтичній та харчовій промисловостях.

Проблематика. На сьогодні біля 50 % всіх медичних препаратів, харчових добавок та лікарських засобів виготовляють з природної сировини, тому актуальним є пошук рослин з високим вмістом БАС, розробка біотехнологій їх одержання і створення композитів, які забезпечують найбільш ефективне використання діючих речовин.

Мета. Вивчення колекції тропікогенних орхідних для виявлення рослин з високим вмістом БАС. Розробка процедури вирощування перспективних видів *in vitro*. Одержання прототипів лікарських засобів на основі рослинних екстрактів, нанорозмірного кремнезему та біополімеру желатину.

Матеріали й методи. Екстракти готували із листя орхідних, як допоміжні компоненти для створення композитів застосовано кремнезем і желатин. Використано спектрофотометричні, хроматографічні, квантово-хімічні, анатомо-морфологічні, фізіолого-біохімічні та біотехнологічні методи.

Результати. Виявлено види орхідей, які містять значну кількість БАС з високими антиоксидантними властивостями; визначено основні компоненти екстрактів орхідних. Одержано стерильні культури відібраних видів та розроблено протоколи їхнього культивування *in vitro*. Виготовлено два типи біоактивних композитів, вивчено взаємодію компонентів екстрактів з поверхнею кремнезему та молекулами желатину. Показано, що композити на основі екстрактів та кремнезему проявляють високу стабільність при зберіганні та уповільнену десорбцію деяких БАС, тоді як матеріали на основі екстрактів, кремнезему та желатину забезпечують поступове вивільнення всіх компонентів екстрактів.

Висновки. Орхідні є цінним джерелом природних антиоксидантів. Розроблені композити є перспективними для виготовлення лікарських форм для тривалого вивільнення антиоксидантів.

Ключові слова: *Orchidaceae* Juss., рослинні екстракти, поліфенольні сполуки, нанорозмірний кремнезем, желатин, біоактивні композити.