

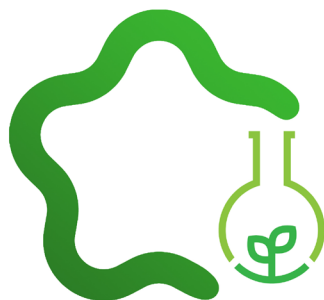
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XXIV Международный Съезд

ФИТОФАРМ 2023

25 – 27 мая 2023 года

Сборник тезисов



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ФЕДЕРАЛЬНОЕ ГОСУДАРСТВЕННОЕ БЮДЖЕТНОЕ ОБРАЗОВАТЕЛЬНОЕ
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Сборник содержит тезисы докладов, представленных в ходе работы XXIV Международного Съезда ФИТОФАРМ 2023 (в смешанном формате) 25 – 27 мая 2023 года. Обсуждаются актуальные вопросы особенностей семеноводства, культивирования, сбора и переработки лекарственного растительного сырья, биотехнологии лекарственных растений (микрклональное размножения, суспензионные и каллусные культуры), проблемам безопасности и эффективности лекарственных средств природного происхождения, стандартизации продуктов природного происхождения, современным технологиям производства натуральных продуктов, этнофармакологии, регуляторным вопросам растительных лекарственных средств и пищевых добавок, химии природных соединений, применению продуктов природного происхождения в пищевой и косметической промышленности.

Сборник тезисов будет интересен специалистам, ученым, студентам, аспирантам и всем заинтересованным в вопросах культивирования, анализа и стандартизации, разработки лекарственных форм, биотехнологии, этнофармакологии и фармакологии, регуляторных аспектов в области фитопрепаратов, лекарственных растений и продуктов их переработки.

Все материалы публикуются в авторской редакции.

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РЕЗОЛЮЦИЯ 24-ого международного Съезда «Фитофарм-2023»

Россия, г. Санкт-Петербург

ФГБОУ ВО «Санкт-Петербургский государственный
химико-фармацевтический университет» Минздрава России
25 – 27 мая 2023 года

Участники Съезда – представители научного сообщества в области фитотехнологии, фитохимии, фармакологии и фармакогнозии ведущих научных школ России, Казахстана, Киргизии, Белоруссии, Вьетнама, Китая, Индии, Южной Африки, Великобритании, Ирана, Египта, Бангладеш, Эфиопии, Эритреи, Шри-Ланки и США, а также представители регуляторных органов и промышленного сектора – отмечают, что в условиях сохраняющейся тенденции к росту потребления фитопрепаратов и биологически активных добавок, возрастания роли правильного питания и профилактической медицины, учитывая смещение фокуса производителей лекарственных средств на отечественные сырьевые ресурсы, идеи межотраслевого (фармация, агротех, фудтех) взаимодействия и разработка фитопрепаратов надлежащего качества из отечественного растительного сырья становятся важнейшим направлением деятельности фитохимического научного и промышленного кластера. Руководствуясь данным тезисом, проблематикой докладов и обсуждений, участники форума считают необходимым отметить, что ключевыми проблемными аспектами и направлениями деятельности являются:

Поиск альтернативных ресурсосберегающих технологий и безопасных растворителей (н/р, из числа «зеленых» растворителей) для комплексного извлечения биологически активных веществ из природного сырья, обусловленное смещением ценностных ориентиров и факторов выбора на новые модели производства и получение продуктов с улучшенными и заранее заданными свойствами.

Расширение биоколлекций культур клеток лекарственных растений, изучение закономерностей накопления вторичных метаболитов и путей их биосинтеза в условиях культивирования *in vitro*; отбор ценных штаммов, в особенности продуцирующих сильнодействующие БАВ (алкалоиды и тритерпеновые гликозиды), для планирования и осуществления рентабельного биотехнологического производства фитопрепаратов.

Разработка подходов комплексной оценки качества растительных продуктов (фитопрепаратов, биологически активных добавок к пище, продуктов специализированного и функционального питания) пригодной для целей кросс-отраслевого применения с использованием риск-ориентированного подхода и с конкретизацией критических параметров (н/р, оценка специфичности методик анализа мультикомпонентных объектов, сочетанное аналитическое влияние компонентов растительных продуктов).

Направленное исследование первичной и вторичной фармакодинамики растительных средств в части, касающейся фармакологических эффектов и механизмов их реализации с целью повышения уровня доказательности эффективности фитопрепаратов.

Научное взаимодействие (совместные проекты, гранты, программы исследований) со странами СНГ и дальнего зарубежья с целью этнофармакологического изучения эндемичных природных объектов различных эколого-фитоценологических зон для поиска источников биологически активных веществ с антибактериальным и противоопухолевым действием.

Поиск новых сырьевых источников, разработка оптимальной промышленной технологии и изучение строения природных полимерных соединений (н/р, полисахаридов различных фракций, в т.ч. пектинов), как систем транспортной доставки АФС, потенциальных лекарственных кандидатов, а также веществ, имеющих мультивекторное применение как в фармацевтической, так и пищевой промышленности в качестве продуктов функционального питания и корригентов органолептических и физических характеристик.

Поиск и выделение в индивидуальном состоянии биологически активных веществ с выраженной антимикробной и противоопухолевой активностью из растений и грибов (в т.ч. пептидов, наряду с нерибосомальными пептидами) с последующей оценкой их фармакологической активности на моделях *in vitro*.

Организационный комитет конференции выражает благодарность спонсорам конференции за поддержку международного научного форума.

Данная резолюция будет передана соответствующим научным институтам, организациям, представителям целевых отраслей промышленности и законодательным структурам для дальнейшего рассмотрения и принятия необходимых мер.

Председатель Организационного комитета
24-го международного Съезда «Фитофарм-2023»

И.А. Наркевич

Key note lectures Phytopharm

ИССЛЕДОВАНИЯ И ФАРМАЦЕВТИЧЕСКИЕ РАЗРАБОТКИ ЛЕКАРСТВЕННЫХ СРЕДСТВ НА ОСНОВЕ ПРИРОДНЫХ ОБЪЕКТОВ СИБИРИ (плeнарный доклад)

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Ключевые слова: природные биологически-активные соединения, растительное сырье, фармацевтическая разработка.

Ученые СибГМУ и ТНИМЦ СО РАН имеют многолетний опыт совместных исследований и разработок в области создания новых лекарственных средств на основе растительных объектов, произрастающих в сибирском регионе. Исследования осуществляются на всех этапах полного цикла фармацевтической разработки.

В докладе будут освещены основные результаты и достижения в области разработки остеогенных, гиполипидемических, противоопухолевых, антитабачных, противогельминтных, иммуностимулирующих и цитопротекторных лекарственных средств на основе индивидуальных природных низко- и высокомолекулярных соединений и представлена схема междисциплинарного непрерывного процесса исследования и разработок от «идеи до клиники», основанная на опыте взаимодействия кафедр фармацевтического и медико-биологического факультетов, а также лабораторий и центра стратегического проекта «Таргетная тераностика» Сибирского государственного медицинского университета.

PEPTIDE-BASED QUALITY CONTROL APPROACH FOR ANIMAL-DERIVED TCM DRUGS

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Abstract. Animal-derived Chinese materia medica contributed a big part of the traditional Chinese medicine system, which accounted for around 1800 plus species among a total of 12408 traditional Chinese medicines. But due to the lack of knowledge of what constitutes the therapeutic principles, the quality control of these types of drugs remained almost mysterious and almost no assay methods were applied to the quality monographs. In a bid to facilitate the elaboration of feasible and scientific quality monograph for the animal-derived drugs in the Chinese pharmacopoeia, peptide-oriented research has been conducted with a few of typical drugs such as Cordyceps, Leeches, Pheretima, Donkey-hide gelatin, among others. For Cordyceps, an untargeted metabolomics strategy based on UPLC-Q-TOF/MS was developed to characterize the metabolites of wild and cultivated Cordyceps and its analogues, counterfeits, and fermented mycelia. Finally, 139 features were screened to distinguish Cordyceps from other varieties, 84 features were used to distinguish wild and cultivated Cordyceps samples, 56 characteristics were obtained to effectively identify analogues, counterfeits, and fermented mycelia of Cordyceps, and 17 of these differential features were identified through database searching. Furthermore, based on the selected features, the automatic model identification of caterpillar fungus species and WOSH & AOSH was realized by using support vector machine, and the accuracy rate was close to 100%. Similarly, profiling methods including database construction, targeted and untargeted metabolomics were applied for Leeches, Pheretima, Pinelliae Tuber for the efficient differentiation of a bevy of analogous species including faked ones. As a result, it demonstrated that the developed method is a quite feasible approach to realize the species differentiation and quantitative determination of peptide meaningful markers.

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**ETHNOPHARMACOLOGY FOR THE DEVELOPMENT OF PHYTOPHARMACEUTICALS:
SYNERGY AND SYSTEM BIOLOGY BASED INTEGRATED APPROACH**

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Ethnopharmacology have been using from the ancient time to manage and treat human ailments. Ethnopharmacology based drug development are gaining global acceptance because of their holistic approaches for therapy and less toxicity. Ethnopharmacology stands for a unique viewpoint for extensive and multidisciplinary research with the integrated approaches for development of newer drug from natural resources especially from Medicinal Plants. Medicinal plants are used in diverse traditional systems of Medicine.

Evidence based validation of the ethnopharmacological claims on traditional medicine is the need of the hour for its globalization and promotion. Many of the world's top-selling drugs were discovered through the study of traditional medicine, such as the anticancer drug Taxol, antimalarial drug Artemisin etc. The validation of drugs developed through ethnopharmacology is crucial to ensure their safety and efficacy. To maintain the quality of herbal drugs proper authentication of plants along with phytochemical analysis and standardization of the desired formulation is essential.

Metabolomics based profiling of herbal extracts and formulations in high resolution through hyphenated technology including LC-MS/MS, GC-MS and NMR providing the possibility of multi-component drug discovery. Combination synergy is a multi-dimensional concept where synergy can be observed from a network pharmacology perspective. This network pharmacology with metabolomics has been proven to be effective in elucidating the mechanisms of action of medicinal plants and complex traditional formulations. This approach will be beneficial for effective evaluation of safety and toxicity of botanical mixtures along with the probable mechanism of action against disease condition. The system biology based approach coupled with modern metabolomics investigations will unfold enormous possibilities for validation and development traditional medicine as an alternative healthcare for the society at large.

CYTOTOXIC POTENTIAL OF *RUTA CHALEPENSIS* FROM LIBYA

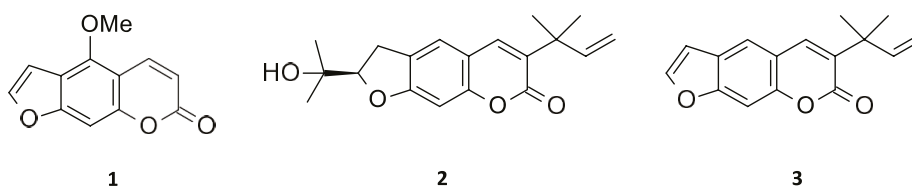
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Cancer is one of the leading causes of mortality and morbidity in humans. Globally, approximately 19.3 million new cancer cases and 10 million cancer deaths were recorded in 2020 [1], and the number of cancer cases is set to increase because of the high level of environmental pollution and unhealthy lifestyle. The currently available cancer treatment options are time-consuming, often ineffective, costly, and suffer from serious side effects. Therefore, the search for new cancer chemotherapeutic agents continues. Plants have been a rich source of anticancer drug molecules, e.g., taxol in *Taxus brevifolia*, and vincristine and vinblastine from *Vinca rosea* [2]. In continuation of our search for new potential chemotherapeutic agents from higher plants [3-8], *Ruta chalepensis* L., which is a well-known medicinal plant from Libya, was subjected to *in vitro* cytotoxicity screening using various human cancer cell lines, e.g., EJ138 (human bladder carcinoma), HepG2 (human liver hepatocellular carcinoma), A549 (human lung carcinoma), MCF7 (human breast adenocarcinoma), prostate cancer cell line (PC3) and a noncancerous prostate cell line (PNT2), followed by bioassay-guided isolation of active principles from active extracts/fractions. Among the extracts, the dichloromethane (DCM) extract was the most active one with IC₅₀ values ranging from 15 to 60 µg/mL. Bergapten (**1**), chalepin (**2**) and chalepentin (**3**) were isolated as the major compounds from the active DCM extract. While bergapten (**1**) was not found to be cytotoxic against the tested cancer cell lines, chalepin (**2**) and chalepentin (**3**) were cytotoxic against the A549 cell line with IC₅₀ = 92 µM and 200 µM, respectively. Chalepin (**1**) also showed cytotoxicity against the E138 cell line (IC₅₀ = 117 µM). The cytotoxicity displayed by *R. chalepensis* was more prominent against selective cancer cells than against normal cells. Cytotoxicity alone may not be an indicator for any plant extracts or compound to be considered to have anticancer properties, but selective cytotoxicity may shed some light on the plausible anticancer property of this plant and its isolated compounds.



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**METABOLOMIC PROFILING OF TRADITIONAL INDIAN MEDICINE
FOR SCIENTIFIC VALIDATION OF TRADITIONAL CLAIMS**

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Keywords: *Metabolomics, Quality control, Traditional Unani Medicine, Ethnopharmacology, Network Pharmacology.*

Abstract. The traditional Unani medicine originated in Greek and developed in India relied on natural healing based on principles of harmony and balances. Similar to other Ayush medicine, Unani medicines are processed as per classical literature and used singly or compounded with other substances to achieve synergistic, antagonistic or detoxifying effects. The Indian traditional medicine (Ayush medicine) have long history of safe use and consists of Ayurveda, Yoga, Naturopathy, Unani, Siddha, Sowa rigpa and Homeopathy systems of medicine.

Modern analytical techniques such as LC-MS, GC-MS and NMR are the most specified techniques in addition to HPLC, UPLC and HPTLC to validate the Ayush medicine for their standardization and authenticity by evaluating their bioactive metabolites followed by network pharmacology studies. Further, high resolution LCMS/MS based fingerprinting and dereplication of metabolites present in bioactive botanicals and in traditional herbal formulations have been used not only for metabolite profiling for quality control but also for mechanistic networking and pharmacokinetics as well as toxicity evaluation of these Traditional medicines. We have used LCMS, GCMS and TLC-MS hyphenated bioautographic techniques for scientific validation of traditional claims of different Traditional Unani medicines used for different chronic disorders through network pharmacology and experimental approaches. It has been observed that metabolomics with network pharmacology may have a great future in quality-based drug development from natural sources as well as for scientific validation of traditional claims.

NATURAL DEEP EUTECTIC SOLVENTS: A NEW CLASS OF GREEN SOLVENTS FOR THE EXTRACTION OF ACTIVE COMPOUNDS FROM NATURAL SOURCES

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Natural Deep Eutectic Solvents (NADES) have been recently developed as an alternative class of ecologically friendly and green class of solvents for the extraction of natural compounds. NADES represents the eutectic mixture of hydrogen donors and acceptors native for live plant cells such as sugars, amino acids, weak organic acids etc.

According to the Scopus database, the number of articles in which NADES were reported as an extraction solvent has increased by 40-50% annually in previous years. NADES are used for the extraction of alkaloids, anthocyanins, carotenoids, saponins, steroids, polysaccharides and other groups of plant cell metabolites.

We have applied NADES for the extraction of seaweeds and terrestrial plants. During primary screening study, we have found some NADES which were effective for the extraction of phlorotannins from brown seaweeds *Fucus vesiculosus* and *Ascophyllum nodosum*. The yield of total polyphenols (TP) in NADES was comparable with the yield of TP in acetone and ethanol. In our recent study, after optimizing extraction conditions, we were able to identify by HPLC-HRMS and MS/MS 32 individual phlorotannins in a NADES (lactic acid:choline chloride; 3:1) extract from arctic *F. vesiculosus*. Interestingly, that NADES were useful for the simultaneous extraction of hydrophilic (ascorbic acid, phlorotannins) and lipophilic (fucoxanthin) compounds from *Fucus vesiculosus*. The NADES provides high stability and preserves the antioxidant activity of the extracts from *F. vesiculosus* during 360 days storage. We have established that NADES did not recover As and Co (concentration <LOQ). Moreover, NADES provided a low recovery (<9%) of Ba, Ca, Fe, Mg, Mn, Sr, and Zn from *F. vesiculosus*. The calculated daily intake of all the elements contained in NADES extracts were less than the daily dose risk estimators. NADES were tuned for the extraction of solidoside, tyrosol, rosavin, rosin, cinnamyl alcohol and total markers (sum of phenyletanes and phenylpropanoids) from *Rhodiola rosea* rhizomes. The ability of NADES to co-extract trace elements during the isolation of glycyrrhizic acid (GA) from the roots of *Glycyrrhiza glabra* and the health risks associated with them was evaluated. Due to the close pKa of lactic acid and GA, the yield of GA in lactic acid-based NADES was higher in comparison with other tested NADES. The recovery of all elements (except Li) by all tested NADES was low (less than 6%). According to calculated metal pollution index, hazard quotient, hazard index, and chronic daily intake all tested licorice NADES extracts were nontoxic and possess no health risk for both ingestion and topical application. We have studied the potential of NADES in the extraction of triterpene saponins from the roots of *Aralia elata*. Twenty triterpene saponins were identified using RP-UHPLC-ESI-QqTOF-MS, in the roots of aralia. Thereby, for 13 metabolites, NADES were more efficient solvents in comparison with the water and EtOH.

In general, NADES as alternative solvents have many advantages, such as environmental friendliness, low flammability, obtained from renewable resources, are suitable for extraction of hydrophilic and lipophilic compounds, improves the stability of extracted compounds; have low toxicity (can be used for food purposes, cosmetics, agricultural chemistry, medicine), do not extract toxic elements from plant materials. High viscosity and non-volatility are limitations for NADES (this requires the development of new technologies that can turn these disadvantages into advantages).

EVIDENCE-BASED ETHNOPHARMACOLOGY – EXAMPLES FROM THE AFRICAN FLORA

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Introduction. South Africa harbours an impressive floral diversity and ranks as one of the most biodiverse countries in the world. Interwoven within this botanical tapestry is a cultural heritage characterised by rich indigenous knowledge systems (IKS) which have moulded one of the oldest healing modalities, African Traditional Medicines (ATM). This unique blend of medicinal plant use and IKS has created a unique research opportunity in ethnopharmacological research.

Aim of the Study. Over the past 20 years, our group has endeavoured to provide a scientific rationale for medicinal plant use through an evidence-based research approach of traditional medicines. Several examples will be presented to demonstrate the challenging yet rewarding workflow to explore the chemistry and biological properties of the ethnomedicinal flora of South Africa.

Materials and Methods. Using various *in vitro* and *in vivo* approaches, complemented by analytical methods and multivariate data analysis, we aim to contribute to the fundamental research base required to convert these botanical assets into tangible consumer products.

Results and Discussion. The various challenges facing translation research and the standardisation of ATMs will be highlighted.

ФАРМАКОЛОГИЯ ПРЕПАРАТОВ ПЕПТИДНОЙ СТРУКТУРЫ НА ОСНОВЕ ГРЕЛИНА**Шабанов П.Д.¹**¹ФГБНУ «Институт экспериментальной медицины», Санкт-Петербург, Россия

Базисные механизмы формирования и поддержания зависимости от психоактивных средств до настоящего времени не решены. Используемые в эксперименте методы (самостимуляция структур головного мозга, самовведение, условная реакция предпочтения места и др.) во многом приближают выяснение физиологических и нейрохимических механизмов, лежащих в основе аддикции. В основе данных методов лежат феномены подкрепления, модулируемые аддитивными веществами пептидной и синтетической природы.

Внимание исследователей механизмов зависимости в последнее время привлекает изучение аномального функционирования эмоциогенных структур мозга, активация которых во многом зависит от центральных механизмов стресса. Известно, что нейропептид кортиколиберин (CRF), опосредующий реакцию на стрессорное воздействие, в головном мозге представлен CRF-содержащими нейронами гипоталамуса и структур параамигдаллярного комплекса, именуемой «системой расширенной миндалины» (экстрагипоталамическая система CRF), которой отводят координирующую роль в осуществлении эмоциональных и стрессогенных реакций (Roik R.O. et al., 2019). Важное значение в запуске последних играют нейромедиаторы дофамин и глутамат, а также ряд нейроактивных пептидов, например, CRF, гипокретин (орексин), грелин, нейрокинин, кинспептин и др. Особенно неясным и противоречивым является вопрос о роли нейропептидов параамигдаллярного комплекса в регуляции подкрепляющих систем мозга.

Обоснованием для выполнения настоящей работы послужили данные о возможном вовлечении пептидов системы грелина и гипокретина в общие и стресс-индуцируемые механизмы подкрепления и зависимости от аддитивных веществ, что подтверждается высокой плотностью рецепторов указанных пептидов в структурах параамигдаллярного комплекса (Tissen I.Y. et al., 2019). Регуляция системы положительного подкрепления во многом связана с активацией чувствительных к ним рецепторов гипокретин (орексин) и грелин. Кортиколиберин вызывает деполяризацию гипокретинергических клеток, а антагонист рецепторов CRF астрессин ее снимает. Следовательно, гипокретинергическая система может быть компонентом центрального ответа на острый стресс, который вызывает активацию CRF.

Другой сравнительно недавно открытый пептид, грелин, не менее чем гипокретин связан с системой CRF в головном мозге. Первоначально грелин идентифицирован в тканях пищеварительного тракта, из которого выделяется в качестве гормона, регулируя потребление пищи. Кроме того, грелин также выполняет нейроэндокринную функцию, воздействуя на рецепторы GHSR, локализованные в головном мозге, участвуя в механизмах подкрепления и пищевого поведения. Кроме того, последние исследования (Suarez A.N. et al., 2019) показали важную роль грелина в физиологической реакции мозга на стресс, поскольку одна из возможных мишеней грелина – это CRF-продуцирующие нейроны, активация которых сопровождается экспрессией гена CRF и *c-fos*-маркера клеточной активации в CRF-продуцирующих нейронах (Cabral A. et al., 2012).

В работе приведен физиологический и фармакологический анализ пептидных систем грелина и CRF головного мозга в механизмах подкрепления и разных видов аддикции (алкоголь, психостимуляторы), моделируемых в экспериментальных условиях. Также рассматриваются вопросы создания и изучения лекарственных средств, блокирующих рецепторы грелина, в качестве средств лечения болезней зависимости.

Круглый стол
Особенности законодательного
регулирувания растительных
лекарственных средств и пищевых добавок

SPECIFICS OF THE PREPARATION OF A REGISTRATION DOSSIER FOR HERBAL MEDICINAL PRODUCTS.

MODULE 3. QUALITY

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Introduction. Both within the Eurasian Economic Union, and in many countries, the registration dossier Common Technical Document (CTD) is a uniform format for presenting the application for registration of medicinal products. It is based on the third, fourth and fifth modules representing the evidence base of major characteristics of a medicinal product such as safety, effectiveness and quality. Module 3, which covers data to assess the quality of the drug at all stages within its life cycle, is an essential part of the registration dossier. Requirements for generation and completion of module 3 of the registration dossier for herbal medicinal products in the EAEU are specific and differ from those accepted in the Russian Federation.

Aim of study. Examine the basic requirements and specifics while presenting the application for module 3 of the registration dossier for herbal medicinal products (HMPs).

Materials and methods. The authors used the information and analytical method of research. Legislative actions regulating registration of medicinal products within the EUAU member countries and legal acts that regulate circulation of herbal medicinal preparations served as research material.

Results and discussion. HMPs have a number of characteristics that differentiate them from synthetic drugs. They contain a set of active biological substances determining their pharmacological action. There exist many factors that affect quality and effectiveness of HMPs, such as growing, harvesting (compounding), primary processing, storage of herbal substances and formulation of medicinal products. Standardization of herbal substances is an important step for their effective use. Owing to it, reproducibility of the declared pharmacological action and safety of HMPs can be ensured. The enumerated characteristics of the HMPs determine the requirements for the scope of data presented in the respective sections of Module 3 of the registration dossier. The Economic Commission of Europe approves several guidelines that explain the provisions and requirements of the Rules of registration and expertise of human medicinal products within the part devoted to HMPs.

Conclusion: all data contained in module 3 of the registration dossier should correspond to the requirements set in the legal acts of the EAEU. This will harmonize approaches of the regulatory authorities of the EAEU country members and ensure completeness and uniformity of preparation of all documentation by applicants.

СПЕЦИФИКА ОТПУСКА РАСТИТЕЛЬНЫХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ В АПТЕКАХ

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Фитотерапия сохраняет свою популярность в России, как и во многих других странах. Природное происхождение фитопрепаратов привлекает людей тем, что ассоциируется с естественностью и безопасностью. В нашей стране сложилась ситуация, когда потребителями и специалистами здравоохранения недооцениваются различия между растительными лекарственными препаратами (РЛП) и биологически активными добавками (БАД) на основе растительного сырья, хотя из них только РЛП одобрены Минздравом для лечения и профилактики заболеваний, а за обращением БАД следит другое ведомство, отвечающее за безопасность продуктов питания, – Роспотребнадзор.

Термин «фитопрепарат» используется применительно к обоим видам продуктов (РЛП и БАД), что затрудняет понимание того, с каким о каком именно продукте идёт речь – о лекарстве или пищевой добавке.

Широко используется слово «аналог», которое применяют производители как воспроизведённых РЛП (дженериков), так и БАД, хотя в именно в последнем случае аналогия имеется лишь в том, что присутствует близкий по композиции состав.

Несмотря на законодательные требования к рекламе, производители БАД используют возможности коммуникации с медицинским и фармацевтическим сообществами для позиционирования своей продукции как аналогов РЛП.

Появляются публикации с результатами клинических исследований, проведенных с БАД, однако далеко не всегда можно заключить, получали ли исследователи одобрение своих проектов, как минимум, от локальных комитетов по этике. Подход редакторов научных журналов к публикации таких статей вызывает вопросы, поскольку именно редакторы призваны следить за соблюдением правил биомедицинской этики в подаваемых рукописях.

Существующая практика маркетинговых договоров между аптечными организациями и производителями лекарств и БАД, а также широко распространившаяся практика выпуска аптечными сетями под собственными торговыми марками (СТМ) БАД с составом «аналогичным» РЛП позволили сложиться ситуации, когда назначенные пациентам врачами РЛП в аптеках заменяются фармацевтами на дженерики или даже БАД. Невольно встаёт вопрос о роли врачебных назначений и фармацевтического консультирования в лечении пациентов.

Выводы. Надежда на исправление ситуации с БАД видится в постепенном переходе врачей на работу в соответствии с клиническими рекомендациями, утверждёнными Минздравом РФ, в которые БАД не смогут попасть. Проведение клинических исследований БАД должно соответствовать надлежащей практике и получать предварительно необходимую этическую и, когда применимо, регуляторную экспертизу. Продвижение БАД и РАП в аптеках зависит от комплекса факторов, в первую очередь, экономических и законодательных.

ОСНОВНЫЕ РЕГУЛЯТОРНЫЕ ПОНЯТИЯ И ПОДХОДЫ К РЕГИСТРАЦИИ РАП В ЕАЭС

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Популярность использования в медицине лекарственных растений в самом разном виде требует от государства выверенной регуляторной политики. Регистрация лекарственных препаратов растительного происхождения в Российской Федерации регламентируется Федеральным законом № 61-ФЗ от 12.04.2010 «Об обращении лекарственных средств». В соответствии с решением Совета Евразийской экономической комиссии № 78 от 03.11.2016 о правилах регистрации и экспертизы лекарственных средств для медицинского применения, регистрации подлежат лекарственные препараты, предназначенные для обращения на общем рынке Союза или на территории одного из государств-членов, а регистрационные досье ранее зарегистрированных в государствах-членах препаратов должны быть приведены в соответствие с требованиями ЕАЭС до 31.12.2025г.

Регистрация лекарственных препаратов для их выведения на рынок государств-членов ЕС регламентируется Директивой 2001/83/ЕС. Для растительных лекарственных препаратов (РАП) предусмотрены несколько возможностей регистрации: регистрация с полным досье, регистрация на основании хорошо изученного медицинского применения, а также внесение в реестр традиционных растительных лекарственных препаратов.

Регистрация РАП в ЕАЭС предусматривает следующие варианты: регистрация как РАП, регистрация как РАП с упрощенным регистрационным досье, а также не исключена возможность регистрации как ЛП с хорошо изученным медицинским применением. Несмотря на то, что во многом законодательные требования по регистрации лекарств разрабатывались с опорой на европейские нормативные документы, Правила ЕАЭС для регистрации РАП имеют, кроме сходств, также и существенные отличия: например, в некоторых случаях требуются локальные клинические исследования или длительный опыт применения (не менее 10 лет с даты первого систематического и документированного применения лекарственного растительного препарата не менее чем в 3-х государствах-членах).

Рабочей группой ЕЭК разработан ряд рекомендательных документов, относящихся к разработке и регистрации РАП, дополняющих Решение № 78.

В докладе представлены сходства и различия терминологии применительно к РАП в ЕС, ЕАЭС и РФ, а также применительно к лекарственному растительному сырью и растительной фармацевтической субстанции.

Выводы. РАП в ЕАЭС имеют больше возможностей регистрации, по сравнению с национальными требованиями РФ. Несмотря на схожесть терминов в законодательстве ЕАЭС и ЕС, они не эквивалентны. Законодательная база ЕАЭС находится в стадии формирования, адаптируясь к реальной ситуации в системе регулирования Союза.

COMPARATIVE ANALYSIS OF REGULATORY REQUIREMENTS FOR HERBAL MEDICINES AND DIETARY SUPPLEMENTS. THE CHOICE IS UP TO BUSINESS

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Keywords: *herbal medicinal products, herbal dietary supplements, marketing authorization in EAEU, the Russian pharmaceutical market.*

Introduction. In 2020, pharmacy sales of herbal products amounted to 435.4 million packages worth 51.3 billion rubles in wholesale prices [1]. These products are presented two categories: herbal medicinal products (HMPs) and herbal dietary supplements (HDSs). HMPs make up the main share of the market, accounting 80% in volume terms and 74% in value terms. However, HDSs are leading in terms of the number of trade name items. The entry into force of the Eurasian Economic Union (EAEU) legislation affected both categories of herbal products and influenced business strategies in their market access.

Aim of study. A comparative analysis of marketing authorization requirements for HMPs and HDSs in accordance with the EAEU legislation, as well as an assessment of impact of regulatory changes on business activity in the field of herbal products approval.

Materials and methods. The study was based on the marketing authorization legislation for HMPs and HDSs, as well as an official information on products registered.

Results and discussion. Transition since 2021 to the regulatory procedure in accordance with the EAEU legislation has complicated the process of forming a registration dossier (RD) for HMPs. Until the end of 2025, there is a transition period to bring the RD for medicines presented on the market into line. Since 2021, the RD for 35 HMPs have been brought into compliance, but this is only those medicines that have a relatively high level of sales. At the same time, during this period about 2 thou HDSs were registered, including those which have a composition like HMPs. Since the entry into force of the EAEU requirements, not a single HMP with an original composition has been approved.

Conclusions. The regulatory model created in accordance with the EAEU legislation, with a general increase in the quality and safety requirements, at the same time does not create the necessary incentives for applying herbal products for marketing authorization as HMPs.

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REGULATION OF HERBAL PRODUCTS IN THE UNITED STATES

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Abstract. America is unique in the world in not recognizing the value of herbal medicine and traditional healing practices in modern health care. Rather, herbal products are regulated as herbal “supplements” that may be used for general health but are strictly prohibited from being promoted for the prevention or treatment of disease. The United States is also unique in being one of the only countries without a nationalized health care system. This creates negative economic barriers to the use of herbal preparations as medicines as approval as a medicine requires substantial financial capital. Despite economic and regulatory barriers, use of herbal supplements continues to grow both among consumers and health care professionals. Herbal products are regulated by the Food and Drug Administration as well as other regulatory bodies, must be manufactured according to Good Manufacturing Practices, and manufacturers of products must maintain an adverse effects reporting program for serious adverse events. This presentation will provide an overview of the regulation of herbal dietary supplements in the United States.

Congress abstracts

DETERMINATION OF TUYON IN WORMWOOD AND EXTRACTION DRUG BASED ON IT

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Introduction. Wormwood – *Artemisia absinthium* L., widely distributed in the European part of the Russian Federation (RF), contains a rich complex of biologically active substances. In medical practice, bitter wormwood grass is used only in the form of infusion and tincture. Development of extraction preparations from wormwood is largely restrained by presence of tuyon and its derivatives in essential oil, which has neurotoxic action [1]. It is known that during extraction, the amount of tuyon increases significantly with an increase in the concentration of ethyl alcohol [2]. This makes the study of the presence of tuyon derivatives in the wormwood and an extraction preparation enriched with flavonoids relevant.

Aim of the Study. Comparative evaluation of tuyon content in essential oil and flavonoid-enriched extraction preparation from wormwood.

Materials and Methods. The work used raw materials – wormwood, collected in 2018-2019, in the Dobryansky district of the Perm Territory during the flowering phase and a dry extract based on it, obtained according to the technology developed by us. Quantitative determination of essential oil was carried out by ОФС.1.5.3.0010.15, method 2, qualitative analysis of tuyon by gas chromatography with mass spectrophotometric detection on an Agilent chromatograph 7890A with a mass spectrometer 5975C as a detector. The obtained mass spectra were compared with library (library: NIST11).

Results and Discussion. The quantitative content of essential oil in the wormwood was $0.60 \pm 0.03\%$. At the same time, the content of tuyon in essential oil for the hemotype of wormwood European part of the Russian Federation is up to 35%. [3]. It has been experimentally proven that 70% ethyl alcohol is the optimal extractant for obtaining an extract enriched with flavonoids from wormwood. A high percentage of ethyl alcohol increases the likelihood of extraction from raw materials and tuyons.

During qualitative analysis of the tuyon, the conditions for chromatographic separation and mass spectrometric analysis were established: non-polar column HP-5ms; evaporator temperature 280 ° C; carrier gas helium 1 ml/min; column temperature: 100 ° C (2 min), 100-290 ° C (at a rate of 20 ° C/min); isothermal mode at 290 ° C – 25 min, registration of mass spectra – by full ion current. Identification of the tuyon was carried out based on a comparison of retention times and full mass spectra.

Comparative analysis of the obtained chromatograms showed the presence of a peak of tuyon with a retention time of 3.48 minutes in the essential oil separated from the raw material of the wormwood. Convergence with library mass spectra is 92%. There is no peak in the dry extract chromatogram in this area.

Conclusions. Comparative analysis shows the content of tuyon in the essential oil of the wormwood. As part of the extraction preparation of wormwood, enriched with flavonoids, this substance is absence.

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EVIDENCE-BASED ETHNOPHARMACOLOGY – EXAMPLES FROM THE SOUTH AFRICAN FLORA

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Southern Africa harbours an impressive floral diversity and ranks as one of the most biodiverse countries in the world. Interweaved within this botanical tapestry is a cultural heritage characterised by rich indigenous knowledge systems (IKS) which have moulded one of the oldest healing modalities, African Traditional Medicines (ATM). This unique blend of medicinal plant use and IKS has created a unique research opportunity in ethnopharmacology. Over the past 20 years our group has endeavoured to provide a scientific rationale for medicinal plant use through an evidence-based research approach of traditional medicines. Several examples will be presented to demonstrate the challenging yet rewarding workflow to explore the chemistry and biological properties of the ethnomedicinal flora of South Africa. Using various *in vitro* and *in vivo* approaches, complemented by analytical methods and multivariate data analysis we aim to contribute to the fundamental research base required to convert these botanical assets into tangible consumer products. The various challenges facing translation research and the standardisation of ATMs will be highlighted.

CHARACTERISTICS OF THE POLYSACCHARIDE COMPOSITION OF CHLORELLA BIOMASS

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Introduction. Currently, chlorella is actively used as a natural source of substances that are in demand in a healthy diet. Among such components of chlorella, proteins, vitamins (A, B1, B2, B6, C, K, PP, E, pantothenic acid, folic acid, biotin, etc.), trace elements, and essential fatty acids are well known. The ease of production of chlorella biomass, along with its composition, allows us to consider it as a very promising object for the production of therapeutic and prophylactic food supplements and a component of vegetarian nutrition. At the same time, along with the rather well-characterized components of this alga, there is relatively little information about the features of its polysaccharide composition. Polysaccharides of medicinal and food plants have been studied for a long time in terms of their biological activity and its correlation with their structure. It is known that polysaccharides are a key component of plant biomass with prebiotic properties; the diversity and structural features of these biopolymers allow them to implement a wider range of useful biological functions (hypoglycemic, regenerative, immunomodulatory, antioxidant, etc.). Identification of the features of the polysaccharide composition of the chlorella cell wall will expand the possibilities of using this alga both in the production of specialized medicinal bioadditives and functional nutrition components.

Aim of the Study. The purpose of this study is to characterize the composition of polysaccharides, retained in various ways in the biomass of chlorella, as a basis for the subsequent development of bioactive additives and health-improving products based on them.

Materials and Methods. Chlorella was grown in Tamiya liquid mineral medium under 12/12 light (light/dark). The biomass separated from the culture liquid was washed with distilled water, acetate buffer and freed from components soluble in organic solvents (ethanol, methanol, chloroform, acetone). Polysaccharides extracted from biomass with NaOAc buffer (pH 5.0), 0.5% ammonium oxalate solution (chelating agent, destroying ionic bonds) and 4M KOH (destruction of hydrogen bonds and swelling of cellulose microfibrils with the release of part of the polysaccharides retained by cellulose). For all obtained fractions, the molecular weight distribution of substances was determined using gel-filtration (Agilent 1260, RID, Shodex OH-Pak 806M) and the monosaccharide composition of TFA-hydrolyzed polysaccharides was determined using anion-exchange chromatography (ICS-6000, IntAmp, CarboPac PA-1). Kallose defined with aniline blue.

Results and Discussion. All obtained polysaccharide fractions (extracted by distilled water, acetate buffer, a 0.5% solution of ammonium oxalate, and 4M KOH) consisted of 2-4 containing polysaccharides (higher molecular weight binding glycans and lower molecular weight callose) and glycosaminoglycans (about 8%). In the KOH fraction, binding glycans and pectins were also detected, and the proportion of glucose in binding glycans and callose increased as the molecular weight of polysaccharides decreased. The proportion of glycosaminoglycans extractable with alkali was extremely low (about 1–4%). Thus, it has been established that chlorella can serve as a source of various polysaccharides, including rather specific structural types and compositions. Further, deeper, more detailed investigation of the identified biopolymer structure and properties will expand the possibilities of their functionalization and application.

**INFLUENCE OF THE STATE PHARMACOPEIUM OF THE RUSSIAN FEDERATION
ON THE QUALITY OF THE MEDICAL PLANT RAW MATERIALS AND DRUGS BASED ON IT**

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Keywords: *medicinal plant materials, State Pharmacopoeia of the Russian Federation, pharmacopoeial articles.*

Introduction. As part of the preparation of the State Pharmacopoeia of the Russian Federation XV edition, pharmacopoeial articles on methods of analysis of medicinal plant materials are harmonized with the monographs of the Pharmacopoeia of the Eurasian Economic Union and the provisions of the world's leading pharmacopoeias. The formation of common approaches and requirements is important for the unification of the quality examination and registration of herbal medicines at the national and regional levels. The **aim of the work** is to create a regulatory framework for the standardization and introduction of high-quality of medicinal plant materials and medicines based on it to the Russian pharmaceutical market. At the first stage, the requirements of all general pharmacopoeial monographs for medicinal plant materials are updated with subsequent updating of pharmacopoeial monographs for medicinal plant materials.

Result and discussion. The main directions of updating the pharmacopoeial articles:

- supplementing the general pharmacopoeial monographs with methods of physical and chemical analysis, taking into account modern requirements, scientific and practical achievements in the field of pharmacopoeial analysis;
- inclusion of new, previously absent in the State Pharmacopoeia of the Russian Federation XIV edition. In total, 32 general pharmacopoeial monographs were updated, 9 new general pharmacopoeial monographs were prepared for inclusion.

Currently, work is underway to update the monographs for medicinal herbs, taking into account the expansion of its nomenclature by including new types of medicinal herbs as part of the recommendations of the working group to develop proposals for the development of production and processing of medicinal and essential oil crops in the Russian Federation, organized at the site of the Federation Council of the Federal Assembly of the Russian Federation Federation.

The development and updating of the pharmacopoeial monographs is a multi-stage process of reviewing draft, which involves leading experts from the *FSBI "SCEEMP" of Ministry of Health of the Russian Federation*, representatives of drug manufacturers and other subjects of drug circulation. Upon completion of the development, the draft pharmacopoeial monographs is reviewed and then reviewed at the specialized standardization sections of the Institute of Pharmacopoeia and Standardization in the Sphere of Medicines Circulation, approved by the Pharmacopoeia Committee and posted on the website of the Russian Ministry of Health for public discussion, after which it is approved by the Council of the Russian Ministry of Health on State Pharmacopoeia.

Feedback. Remarks, comments and proposals on the content of the pharmacopoeial monographs on of medicinal plant materials are accepted at all stages of development/updating of the pharmacopoeial monographs by e-mail: **pharmacopoeia@expmed.ru**

**SPECTROPHOTOMETRIC DETERMINATION OF THE POLYSACCHARIDES CONTENT
IN PHARMACOPOEIAL MEDICINAL PLANT RAW MATERIALS****Bokov D.O.** (ORCID: 0000-0003-2968-2466)^{1,2}, **Samylina I.A.** (ORCID: 0000-0002-4895-0203)¹¹ Sechenov First Moscow State Medical University

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E-mail: bokov_d_o@staff.sechenov.ru**Keywords:** *polysaccharides, medicinal plant raw materials, spectrophotometry.*

Introduction. In our work, we consider the main procedures that are used in the pharmacopoeial spectrophotometric (SPh) method for assessing the content of polysaccharides (PSC) in medicinal plant raw materials (MPRM), its advantages and disadvantages. The pharmacopoeial (according to State Pharmacopoeia of Russian Federation XIV ed. – SPRP XIV ed.) medicinal plant raw materials (MPRM) containing PSC include kelp thallus, flax seeds, broadleaf plantain leaves, violet herb, three-part beggarticks herb, elfdock rhizome and roots, linden flowers, burdock roots, coltsfoot leaves, marshmallow roots [1]. The gravimetric (weight) method is used to determine the total content of PSC in flax seeds, violet herb, three-part beggarticks herb, broadleaf plantain leaves. Meanwhile, the SPh method, which is described in other pharmacopoeial monographs (PM), has both a number of advantages and disadvantages [2].

Aim of the Study. Consider the prospects for further development of spectrophotometric methods for assessing the content of polysaccharides in pharmacopoeial MPRM.

Materials and methods. The work used the materials of our own research, as well as the analysis of regulatory documentation, including drafts of pharmacopoeial monographs.

Results and discussion. The SPh method is more rapid than the gravimetric method, and its labor intensity is lower. The main disadvantage of SPh techniques is the rather low specificity, and therefore overestimated results can be obtained. The SPh procedures used in the SP RF XIV assume the use, mainly, of standard samples (SS) of glucose and fructose. In some PM drafts the conversion is carried out for xylose and galacturonic acid, but these SS are less accessible in analytical laboratories. In the General PM “Determination of sugars by the SPh method”, in addition to the reaction with picric acid, anthrone and orcin are also included. Most of the PM for MPRM include methods with picric acid (conversion to glucose, etc.). From the point of view of reproducibility, more accurate results are obtained by anthrone and orcin methods compared to the picric method, which does not always ensure the convergence of results. The simplest SPh variant involves complete acid hydrolysis of PSC without preliminary removal of free carbohydrates (CH). As a result, the indicator «the sum of PSC and reducing sugars in terms of ...» is obtained. Free CH in the MPRM of the leaves and herbs groups are contained in a small amount (up to 2%), so the contribution to the total indicator is low. But in the case of subterranean organs, where the free CH content can accumulate in greater quantities, the question arises of removing free CH.

Conclusions. Thus, it should be noted that the development and validation of new spectrophotometric methods for inclusion in the State Pharmacopoeia is an urgent task.

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ADAPTOGENS: PROBLEMS OF CLASSIFICATION, SAFETY AND SELECTION
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Abstract. The range of medicines of the group “General tonics and adaptogens” was studied, problems of classification were identified and recommendations were given for adjusting the algorithms of pharmaceutical counseling.

Keywords: *adaptogens and general tonic preparations, herbal adaptogens, pharmaceutical consulting*

Introduction. In recent decades, despite the formation of new health-saving strategies aimed at creating an understanding among the population of the importance of a healthy lifestyle and proper nutrition, timely detection and prevention of diseases, medicines designed to increase mental and physical performance, general nonspecific body resistance remain extremely popular [1], general strengthening, tonic, adaptogenic action.

Aim of study. Studying the range of medicines of the group “General tonics and adaptogens”, identifying consumer preferences and important components of pharmaceutical consulting.

Materials and methods. The study of the assortment was carried out using the electronic pharmacological reference books Vidal, RLS, the Internet resource “AnalitPharmacia” and the State Register of Medicines (as of 01.01.2023). Consumer preferences were identified using an online survey (duration 2 months), the sample was 100 people.

Results and discussion. The analysis of information resources revealed the problem of ambiguity in assigning drugs of the category under study to classification groups, which significantly complicates the search for therapeutic analogues and presents a serious problem when replacing a drug in pharmaceutical counseling. On the Russian pharmaceutical market, the preparations of the General toning agents and adaptogens group are represented quite widely, herbal preparations predominate, which, according to consumers, is due to their effectiveness. It was revealed that in the pharmaco-therapeutic group “Adaptogenic agent” there are drugs of the opposite direction of action, both general tonic and hypnotics, as well as a number of drugs exhibit additional effects that expand the list of indications for use and at the same time sometimes determine contraindications. The greatest demand falls on classical phytoadaptogens (eleutherococcus, ginseng, magnolia vine, etc.), however, when conducting pharmaceutical counseling, it is necessary to exclude comorbidities and contraindications.

Conclusions. The modern classification of drugs in the group “General tonics and adaptogens” in the Russian Federation is imperfect and needs to be adjusted. In pharmaceutical counseling, it is necessary to optimize the choice of the drug of the study group, both taking into account the “drug profile” and taking into account the “patient profile”.

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METHODS FOR CATALASE ACTIVITY DETERMINATION IN PLANT CELL CULTURES

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Introduction. Antioxidant enzymes (catalase, superoxide dismutase, peroxidase) are part of the first line of cell protection against excessive accumulation of reactive oxygen species. [1] The content of components in cells that exhibit antioxidant activity is recognized as an important indicator of the vital status of plant tissue culture.

Aim of the Study. The objective of this study is to compare different methods for catalase activity determination and select the optimal technique for further work.

Materials and Methods. Measurement of catalase activity in the plant cultures of *Cichorium endivia*, *Scutellaria baicalensis* Georgi, *Panax quinquefolius*, are being cultivated in a tissue culture laboratory at St. Petersburg State University of Chemical and Pharmaceutical Sciences, was carried out by titrimetric and spectrophotometric methods [2].

Results and Discussion. Titrimetric method for determination of the catalase activity has demonstrated a number of disadvantages, namely low sensitivity, necessity of significant amount of material, so it can't be used in further work.

It was found, that it is necessary to use concentrated supernatant for spectrophotometric evaluation of catalase activity within the determination limits of spectrophotometer LEKI ss 1207. The method of catalase activity evaluation with $(\text{NH}_4)_2\text{MoO}_4$ is usually used for animal material. The activity of catalase is extremely high; therefore, the homolysate is diluted 1000 times or more. Solution comes out almost transparent and its optical density can be neglected. In the case of plant tissue cultures, a solution with a dilution of 1:10 or 1:1 was used in the experiments. At low dilution, solution demonstrates intensive coloration and affects the optical density measurement. Since the supernatant, according to the method, is not contained in the blank sample, the difference in optical density between the experimental and blank samples will be less than the theoretical one up to negative values. To avoid an error, the supernatant is added to all cuvettes, but the supernatant is added to the blank sample only after the solution in the cuvette is incubated with $(\text{NH}_4)_2\text{MoO}_4$, namely immediately before value fixation on spectrophotometer. In this case, no water is added to the blank sample.

Conclusions. The modified method for plant tissue cultures catalase activity determination was developed. This modified technique allows obtaining a more accurate result both in plant and animal material.

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MICROCLONES OF *SCUTELLARIA BAICALENSIS* GEORGI AS A SOURCE OF FLAVONOIDSBronskikh E.D.¹, Pivovarova N.S.¹¹Saint-Petersburg State Chemical and Pharmaceutical University

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Introduction.

Scutellaria baicalensis Georgi (*S. baicalensis*) contains a number of flavonoids valuable for humans – these are baicalin, baikalein, warrior, scutellary [1]. However, the ranges of its growth in Russia are limited, and its natural reserves are decreasing, the species is included in the lists of rare plants [2]. Therefore, the study of alternative ways of its cultivation for the production of valuable biologically active substances becomes relevant.

Aim of the Study. Grow microclones of *S. baicalensis* and prove their ability to synthesize flavonoids.

Materials and Methods. Explants for *in vitro* culture prepared by germination of *S. baicalensis* seeds under aseptic conditions on Murashige-Skoog (MS) medium. To prevent contamination, the seeds treated with a 3.5% sodium hypochlorite solution with an exposure time of 10 minutes. Microclones were cultured on MS containing half the major salts (1/2 MS). Plants grown in culture vessels at + 25 ° C, photoperiod day/night – 16/8 h, illumination 6000 lux, relative air humidity 60-70%.

To evaluate the presence of flavonoids in the roots of microclones, the plant material was triturated in a mortar with 70% ethanol in a ratio of 1:10, then macerated for 48 h. and centrifuged at a 7000 g of 15 min., then standard qualitative reactions to this group of substances were carried out.

Results and Discussion. To increase secondary explants from the obtained aseptical seedlings of *S. baicalensis*, epicotyl separated and transplanted into 1/2 MS. After 2 months of cultivation, multiple shoot formation observed on such a medium. The average number of shoots was 15,6±0,5 pcs. The average length of shoots –13,8±0,7 mm. It noted that when culturing *S. baicalensis* microclones on culture medium containing 30 g/l sucrose, no process of root formation observed. With a decrease in the concentration of carbon nutrition to 20 g/l, but with the preservation of the rest of the component composition of the culture medium, the development of the root system recorded in microclones. The average number of roots per 1 plant was 3,3±0,5 pcs.

The results of qualitative analysis of extraction obtained from the roots of *S. baicalensis* microclones for flavonoids are presented in Table 1.

Table 1 – Qualitative analysis of the extract for flavonoids

Qualitative reaction	Observed reaction effect	Proposed compounds
10% FeCl ₃	Green color solution	Flavonols
10% Pb(CH ₃ COO) ₂	Formation of bright yellow precipitate	Flavons, Chalcones, Aurones
10% NH ₄ OH	Yellow color solution	Flavones, Flavonols, Flavanones

Conclusions:

1. When culturing *S. baicalensis* microclones on 1/2 MS and 20 g/l of sucrose, multiple shoot formation and root formation are observed.
2. It was revealed that the roots of *S. baicalensis* microclones contain flavons, flavonols, flavanones, chalcones, aurones.

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ПОИСК ИСТОЧНИКОВ ТИМОХИНОНА

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298500, ул. 15 апреля, 37, г. Алушта, Российская Федерация,**E-mail:** Burtsevaev2009@yandex.ru**Ключевые слова:** тимохинон, *Nigella sativa* L., эфирное масло.

Актуальность. Обнаружение у тимохинона противоопухолевых, противовоспалительных, иммуномодулирующих, гипотензивных, антидиабетических, гиполипидемических, антигистаминных, гепато- и нефропротекторных, антиоксидантных, антиишемических и радиозащитных свойств делает данное вещество перспективным для лечения множества заболеваний [1]. Известно, что данный хинон накапливается в *Tetraclinis articulata*, *Eupatorium cannabinum*, *Juniperus cedrus*, растениях рода *Thymus*, *Satureja*, *Monarda*, *Nepeta* [2]. Тимохинон является мажорным соединением черного тмина (*Nigella sativa* L.) [3], обосновывая его выбор как источника данного вещества.

Цель. Изучить содержание эфирного масла и тимохинона в семенах *N. sativa* L., произрастающего в различных странах.

Материалы и методы. На накопление биологически активных веществ влияет множество факторов, одни из них климатогеографические. Объектами исследования были семена *N. Sativa*, заготовленные в различных странах: РФ (Крым), Индия, Сирия, Египет и Турция. Эфирное масло получали паровой дистилляцией по методу Далматова. Исследование компонентного состава эфирного масла осуществлялось методом газовой хроматографии на хроматографе «Кристалл 2000М» с пламенно-ионизационным детектором.

Результаты. Выход эфирного масла из семян *N. sativa* L. и содержание в нём тимохинона представлено в таблице.

Таблица – Содержание эфирного масла и тимохинона в семенах черного тмина

Страна культивирования	Турция	Сирия	Египет	Индия	РФ, Крым
Выход эфирного масла, %	0,00	<0,01	0,11±0,01	0,44±0,03	0,48±0,02
Содержание тимохинона в эфирном масле, %	-	-	2,5	14,64	14,85

Как видно из таблицы максимальное содержание тимохинона в семенах черного тмина отмечено в образцах из Индии и Крыма.

Выводы. Выход эфирного масла из семян *N. sativa* L. и содержание в нем тимохинона зависит от климатогеографических условий и является наиболее высоким в сырье растений, культивируемых в Индии и Крыму.

Информация о финансировании. Исследование выполнено согласно соглашению с Министерством промышленности и торговли РФ № 020-11-2021-1073 (идентификатор 0000000002021РРК0002).

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ХИМИЧЕСКИЙ СОСТАВ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ГИДРОЛАТОВ

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Ключевые слова: гидролат, антибактериальная активность, антиоксидантная активность/

Актуальность. Всё большую популярность приобретают продукты переработки растительного сырья. Особую ценность представляют продукты эфиромасличного производства – ароматные воды или гидролаты. Гидролаты широко используются в качестве косметических средств, поскольку содержат ряд биологически активных водорастворимых компонентов эфирного масла, но в отличие от последнего имеют более мягкое воздействие на кожу, что позволяет их использовать их в чистом виде [1].

Цель. Изучить химический состав, антибактериальную и антиоксидантную активности гидролатов.

Материалы и методы. В качестве объектов исследования использовались гидролаты производства АО «АЭМСЗ», полученные из растений: *Lavandula angustifolia*, *Hyssopus officinalis*, *Salvia officinalis*, *Rosmarinus officinalis*, *Rosa damascena* x *Rosa gallica*. Анализ состава проводили методами ГЖХ [2]. Антибактериальные свойства гидролатов изучали на рекомбинантных тест-бактериях *E.coli* (pXen-lux) и на морских светящихся бактериях *Aliivibrio fischeri* F1. Изучение антиоксидантного действия проводилось методом Fe³⁺-индуцированного перекисного окисления липидов суспензии яичных липопротеидов *in vitro* [3].

Результаты. Было выявлено, что гидролат шалфея содержит α- и β-туион, β-карнофиллен, α-терпинеол; гидролат лаванды – камфен, линалоол, линалацетат, гераниол, геранилацетат; гидролат розмарина – камфен, 1,8-цинеол, β-пинен; гидролат розы – фенилэтанол, гераниол, цитронелол, нерол; гидролат иссопа – пинокамфон, изопинокамфон, спатуленол, β-карнофиллен. Антибактериальные свойства исследуемых объектов проявлялись в ингибировании бактериальной люминесценции тест-бактерий на 10%, 44% и более 80% под действием гидролатов розы, лаванды и иссопа, соответственно. При изучении антиоксидантного действия наблюдалась динамика накопления продуктов свободно-радикального окисления липидов, которая в присутствии гидролатов иссопа и розмарина снизилась на 40 и 36% соответственно, в сравнении с контролем.

Выводы. В результате исследований установлено, что исследуемые гидролаты обладают выраженными антибактериальными свойствами. Также, были выявлены антиоксидантные свойства гидролатов *Hyssopus officinalis* и *Rosmarinus officinalis*. Перспективами дальнейших исследований является разработка лекарственных и косметических средств на основе гидролатов вышеуказанных эфиромасличных культур.

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**DETERMINATION OF THE MARKER COMPOUNDS
FOR STANDARDIZATION OF PECTORALES SPECIES NO. 2**

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To improve approaches to the standardization of a mixture herbal product Pectorales species No.2 (PS No.2), the authors used pharmacopoeial and non-pharmacopoeial methods for the determination of marker compounds.

Keywords: *Pectorales species No.2, standardization, polysaccharides, flavonoids, glycyrrhizic acid.*

Introduction. PS No.2 is a mixture herbal product, the infusion of which exhibits expectorant and anti-inflammatory effects. PS No.2 components are represented by coltsfoot leaves (40%), plantain leaves (30%) and licorice roots (30%). These components are included in the State Pharmacopoeia of the Russian Federation XIV ed. Main biologically active compounds of PS No. 2 are polysaccharides, flavonoids, glycerrisic acid [1,2].

Aim of the study. Assessment of the quality of PS No. 2 using pharmacopoeial and non-pharmacopoeial methods, and the search for alternative approaches to the standardization of PS No. 2 through the selection of specific markers.

Materials and methods. Qualitative identification of marker compounds was carried out using pharmacopoeial and non-pharmacopoeial methods (chemical reactions, TLC, ATR IR, UPLC-UV-MS). Quantitative determination of the amount of polysaccharides was determined by the pharmacopoeial method. The content of reducing sugars in terms of glucose was determined by the developed and validated method of spectrophotometry with anthrone reagent. The content of sum flavonoids in terms of rutin was determined spectrophotometrically according to the pharmacopoeial method. The content of glycyrrhizic acid was determined using the developed and validated method of spectrophotometry.

Results and discussion. Qualitative identification methods confirmed the presence of polysaccharides and sugars (galacturonic acid, reducing sugars), flavonoids (quercetin, isoquercetin, kaempferol, rutin etc.) and 18 β -glycyrrhizic acid. The content of polysaccharides was 12.86-13.52%. The content of the amount of reducing sugars (in the composition of polysaccharides) in terms of glucose was 1.89-3.56%. The content of the sum of flavonoids in terms of rutin was 1.45 – 2.82%. The content of glycyrrhizic acid was 3.49 – 5.44%.

Conclusions. These approaches to standardization can be used as the basis for the development of a pharmacopoeial monograph for PS No. 2.

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SAFETY OF GINSENG SUSPENSION CULTURE

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Introduction. The safety of culture tissue and suspension tissue may vary in comparisons with the original plants. In this study we investigated the safety of standardized ginseng suspension culture (SC).

Aim of the Study. The aim of this study is to evaluate the safety of SC by using toxicological, biochemical, hematological and histomorphological approaches at the different levels of biological organization of the body.

Materials and Methods. We used the Russian standard recommendations “Medicines for medical applications. Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals” for safety investigation. The protocol of investigation meets ethical requirements of Ethic Committee of Sechenov University.

Results and Discussion.

Acute toxicity of SC in rats is 750 ml/kg per os, what is very close to the toxicity of natural plant. Determination of chronic toxicity at doses of 0.6 and 6 ml / kg with oral administration of SC did not reveal any serious deviations in all doses. The biochemical analysis shown the deviations in serum concentration of alanine aminotransferase from $48,11 \pm 3,24$ UI/L in control to the $58,90 \pm 7,49$ UI/L in the chronic administration of SC during 6 months in a dose 6 ml/kg per os in mice ($P < 0,05$). The analysis of this deviation indicates a correlation with the data of histomorphological examination. But this deviation is valid only for very high dose of SC -6 ml/kg, which exceeds therapeutic doses in 100 times and parameters of deviation are inside the standard physiological interval for mice[1]. Meanwhile the Bromsulfalein test in dose of SC 6 ml/kg has shown a normal physiological function of liver. In the dose 0,6 ml/kg of SC there is no significant differences in serum concentration of alanine aminotransferase between control (95% CI $48,11 \pm 3,24$ UI/L) group of mice and experimental mice (95% CI $51,40 \pm 12,74$ UI/L). There are no difference between experimental and control groups in histomorphological data in the dose 0,6 ml/kg also. Summing up we can conclude, that the SC has a very low acute toxicity in mice and rats, wide range of therapeutic interval. A comprehensive safety study of SC in the dose 0,6 ml/kg per os, including investigations in toxicological, biochemical, electrophysiological, hematological and histomorphological parameters, did not reveal any negative effect of the drug on the body of experimental animals (95% CI).

Conclusion. SC is a safe biosimilar of natural Ginseng in the chronic experiment in dose 0,6 ml/kg per os.

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STATISTICAL OPTIMIZATION OF SCUTELLARIA BAICALENSIS CELL BIOMASS EXTRACTION PARAMETERS WITH A VIEW TO INCREASING THE YIELD OF POLYPHENOLIC COMPOUNDS**Danilova A.A.¹, Danilov L.G.², Pivovarova N.S.¹**¹Saint-Petersburg Chemical and Pharmaceutical University of the Ministry of Health Protection of the Russian Federation
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Introduction. Extraction is a complex mass transfer process involving the separation of biologically active substances (BAS) from the cellular matrix by selective solvent. BAS diffusion depends on a large set of factors, such as the extractant essence, the duration of the process, the ratio of the raw material to the solvent (hydromodulus), etc. [1]. The concurrent influence of several variable factors results in a quite complicated and time-consuming process in terms of interest molecule extraction. Consequently, the use of statistical experiment planning methods is expedient for increasing the yield of active compounds and reducing the time costs.

Aim of the Study. We focused on the optimization of extraction parameters by means of the one-way and two-way analysis of variance (ANOVA) for polyphenolic compounds accumulated by *Scutellaria baicalensis* biomass.

Materials and Methods. Dry biomass obtained by separation of loose callus from the agarized substrate with subsequent desiccation to constant weight in a dry-heat oven at a temperature of 50 ± 5 ° C. The dry biomass was extracted further using three types of solvents: 50%, 70%, and 80% ethanol (EtOH).

Determination of the extractant efficiency was performed according to a previously created experiment matrix with varying factors (solvent concentration, hydromodulus, and exposure time). We carried out the experiment in 10-fold recurrence for each combination of factors. The total sample volume was 270 samples. The influence of the solvent was estimated by spectrophotometric method. Baicalin, the major component, has a pronounced maximum absorption in the long-wave area at 275 nm, so we applied this value as an analytical wavelength [2]. Statistical study of factor combinations was made using one-way and two-way ANOVA [3]. Tukey's post-hoc test was performed to detect intergroup differences. All statistical analysis was conducted by using the R language.

Results and Discussion. According to the one-way-ANOVA, hydromodulus has a significant effect ($F = 24.631$, p -value = $1.524e-10$) on the diffusion of BAS. The most pronounced effect is in the 1:10 hydromodule. The two-way ANOVA was performed by further considering the exposure time and alcohol concentration. We found the greatest difference in optical density was related to the type of extractant, while the effect of extraction time was expressed to a lesser extent. As a result of this analysis, we can conclude the fact that the combination of extractant type and extraction time significantly affects the average optical density ($F = 3.0540$, p -value = 0.0213367 , $df_1 = 2$, $df_2 = 2$). The following statistically significant differences were found between the 80% EtOH-168 h. and 70% EtOH-168 h. groups. (p -value = 0.00355), 80% EtOH-168 hr and 70% EtOH-240 hr. (p -value = 0.00990). Visualization of the Tukey's post-hoc test indicated the effectiveness of the factors combination: ethyl alcohol 70% and infusion time 240 h. The obtained data are confirmed by the highest values of optical density in the extraction with the specified parameters.

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**CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY
OF ESSENTIAL OILS FROM RESIN OF COMMIPHORA SPECIES****Dinku W.¹, Park S.B.², Jeong J. B.², Jung Ch.^{3,4} and Dekebo A.^{1,3,5}** (ORCID: 0000-0003-4767-606X)¹Department of Applied Chemistry, Adama Science and Technology University, Ethiopia²Department of Medicinal Plant Resources, Andong National University, Andong 36729, South Korea³Agricultural Science and Technology Research Institute Andong National University, South Korea⁴Department of Plant Medicals, Andong National University, Andong GB 36729, South Korea⁵Institute of Pharmaceutical Sciences, Adama Science and Technology University**E-mail:** amandekb@gmail.com

Introduction. There are several *Commiphora* species belonging to the family Burseraceae in Ethiopia. Ethnobotanical information gathered from local people revealed that the resins of these species enjoy a wide array of traditional uses such as human medicine, to treat maladies of cattle and as insect repellents. In some instances, the fruits, resins and other parts of the plants are used as food additives and as chewing gums. Thus, these resins are of considerable medicinal, cultural and economic significance.

Aim of the Study. The aim of this study was to analyze the chemical composition and evaluated anti-inflammatory activities of four resin oils extracted from botanically identified *Commiphora* species.

Materials and Methods. Essential oils (EOs) were prepared by the hydro-distillation technique from the resins of four *Commiphora* species and analyzed by GC-MS. We investigated the anti-inflammatory effects of EOs in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophages by measuring nitric oxide (NO). The effect in mRNA or protein level after EO treatment were evaluated by RT-PCR and Western blot analysis, respectively.

Results and discussion. Major constituents of EOs were α -copaene (22.71%), β -caryophyllene (28.03%) and β -caryophyllene oxide (13.89%) for *C. sphaerocarpa*; α -pinene (29.1%) for *C. africana*; hexadecane (14.1%) for *C. habessinica* and δ -cadinene (31.5%) for *C. schimperi*. Among four *Commiphora* species, *C. sphaerocarpa* EO demonstrated a significant inhibition of LPS by $27.2 \pm 3.6\%$ at 10 $\mu\text{g}/\text{mL}$ and $62.3 \pm 5.2\%$ at 20 $\mu\text{g}/\text{mL}$.

C. sphaerocarpa EO inhibited LPS mediated iNOS over expression in both protein and mRNA level with dose dependent manner. It inhibited phosphorylation of ERK1/2, p38, ATF2. The enhanced anti-inflammatory activity of the EO of the plant was due to HO-1 expression by ROS dependent Nrf2 activation in RAW264.7 cells. These findings indicate *C. sphaerocarpa* EO inhibits the pro-inflammatory responses by inhibiting MAPK/ATF2, and triggering ROS/Nrf2/HO-1 signaling.

Conclusions. Therefore, *C. sphaerocarpa* EO could have potential for useful therapeutic candidate preventing and treating inflammatory diseases.

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ESSENTIAL OILS OF RARE *ARTEMISIA* SPECIES PLANTS OF BURYATIAN FLORA

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Introduction. The Republic of Buryatia is a part of the Baikal unique ecosystem with rare and endemic plant species. *Artemisia jacutica* Drob. is an East Siberian endemic used in Yakut traditional medicine to treat gastrointestinal diseases. In addition, it is a valuable source of chamazulene, which has anti-inflammatory and antioxidant properties [1]. Another species, *Artemisia rutifolia* is a relict species, which grows in Afghanistan, Kazakhstan, Kyrgyzstan, Mongolia, Nepal, Pakistan, Russia (Western and Eastern Siberia), Tajikistan, and Western Asia. It can serve as a reliable natural source of essential oils (EOs) [2].

Aim of study. To investigate the chemical composition of the EOs of *A. jacutica* and *A. rutifolia*, growing in the Republic of Buryatia (Russia), and to study their antimicrobial and antiradical activities.

Materials and methods. The objects of the study were the aerial parts of *A. jacutica* and *A. rutifolia*, collected in 2018-2019 and 2022, respectively, in the Republic of Buryatia, during the vegetation period. The component composition of EOs was determined by gas chromatography-mass spectrometry (GC-MS). Principal component analysis (PCA) method was applied to the contents of EO components. The antiradical activity of EOs was determined by the DPPH test (using a stable radical, 2,2-diphenyl-1-picrylhydrazyl). The antimicrobial activity of the test samples was determined by the technique of diffusion in dense nutrient media.

Results and discussion. EOs of *A. jacutica* were dark blue liquids with a characteristic odor, the oil yield was 0.9, 1.4, and 0.7 % in the budding, flowering, and fruiting phases, respectively. A total of 51 components were identified in EO samples. The highest content of chamazulene (47.77%) was observed in a flowering phase. Two chemotypes of *A. jacutica*, “Yakutian” and “Buryatian”, were distinguished according to the oil composition using PCA. The essential oil exhibited antiradical activity, IC₅₀ was 49.47 µL/mL.

As for another species – *A. rutifolia*, the yield of EOs was 1.82%. 40 components have been identified, most of which were represented by mono- and sesquiterpenoids. The dominant components were 4-phenyl-2-butanone, 1,8-cineol, camphor, terpinen-4-ol, 4-phenyl-2-butanol, *a*-terpineol, *a*-methyl-benzenepropanol acetate, bicyclogermacrene, germacrene D. Monoterpenes, especially the oxygenated ones (46.17%), made up the largest proportion of all components. Using PCA to compare our own and literature data on the content of major components of *A. rutifolia* EO, it was shown that these EOs can be conditionally divided into “Tajik” and “Buryat–Mongol” chemotypes. The results indicated the greatest antimicrobial activity of *A. rutifolia* EO against Gram-positive bacteria (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus cereus*) and fungi (*Aspergillus niger*, *Candida albicans*), with pronounced activity against *Aspergillus niger*. Also, it was found that the EO has high antiradical activity, the IC₅₀ value was 17.55 µL/mL.

Conclusion. *A. jacutica* and *A. rutifolia* are promising species of Asteraceae family. Thus, the high antiradical activity of the EOs, together with a pronounced antimicrobial activity, allows us to consider these species as promising raw materials for the pharmaceutical and cosmetic industries.

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THE STUDY OF ORGANIC ACIDS OF JUNIPER NEEDLES

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Introduction. Organic acids are one of the most common groups of compounds found in plants. They are participants in important biochemical processes, being the precursors of many biologically active substances. A lot of organic acids have their own pharmacological activity, which causes an increase in the interest of researchers in studying the qualitative composition of organic acids and assessing their total content. Juniper needles have traditionally been used in Russia as a remedy for scurvy, but data on the study of organic acids in this type of raw material are presented in insufficient volume. Given the wide range of pharmacological activity characteristic of juniper needles, studies aimed at studying the composition of biologically active substances of this raw material are relevant and promising.

Aim of study. The purpose of this work is to study the composition and quantitative assessment of the content of organic acids in juniper needles.

Materials and methods. The object of the study was juniper needles harvested from cultivated plants in the Botanical Garden of the I. M. Sechenov Moscow State Medical University, as well as from wild shrubs growing in the middle tier of the mixed forest of the Tver region. The harvested raw materials were dried by air-shadow drying and crushed to the size of particles passing through a sieve with a hole diameter of 2 mm. Qualitative analysis of organic acids was carried out by HPLC using a highly efficient liquid chromatograph "Gilston" (France), metal column 6.5 x 300mm ALTECOA -1000 Organic Acids; a solution was used as the mobile phase 0.005 M sulfuric acid solution; the eluent was supplied at a rate of 3 ml/min, the analysis time was 60 minutes, detection was carried out by a UV detector at a wavelength of 190 nm. The total content of organic acids in the studied raw materials was estimated by the method of alkalimetric titration, using 0.1M sodium hydroxide solution as a titrant, the indicator was a mixture of phenolphthalein and methylene blue solutions.

Results and discussion. During chromatographic analysis, individual peaks of carboxylic acids were identified by the addition of standard solutions. In the studied samples of juniper needles, 9 carboxylic acids were found, among which oxalic, citric, malic, ascorbic and succinic acids were identified. The assessment of the total content of organic acids in terms of malic acid showed comparable results for the studied samples. So, for juniper needles cultivated in the Botanical Garden, the content was 2.38%, for wild samples 2.76%. Taking into account the presence of the most important carboxylic acids in juniper needles included in the Krebs cycle, further research of raw materials and the development of norms for the content of organic acids in juniper needles, taking into account the peculiarities of plant growth in different climatic zones, is promising.

Conclusion. During HPLC analysis, oxalic, citric, malic, ascorbic and succinic acids were identified in extracts from the needles of juniper. The analysis of the quantitative content of organic acids was carried out by the method of alkalimetric titration, the total content of organic acids in terms of malic acid was 2.38% for cultivated and 2.76% for wild-growing raw materials.

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IDENTIFICATION OF FLAVONOID AGLYCONES OF FABACEAE FAMILY PLANT EXTRACTS WITH ANTI- α -AMYLASE ACTIVITY BY TLC, MALDI-TOF-MS, AND LC-MS/MS

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Abstract. A combination of TLC-bioautography, MALDI-TOF-MS, and LC-MS/MS methods was used to identify flavonoids with anti- α -amylase activity in extracts of *L. pratensis* L. (herb), *L. polyphyllus* L. (fruits), *T. lanceolata* R. Br. (herb), and *S. japonica* L. (buds). Results of the study revealed that the flavonoids apigenin, luteolin, formononetin, genistein, and kaempferol display marked anti- α -amylase activity.

Keywords: anti- α -amylase activity, flavonoids, MALDI-TOF-MS, LC-MS-MS.

Introduction:

Experiments showed that flavonoids can inhibit α -amylase [1] and contribute lowering postprandial blood glucose levels [2]. Fabaceae plants are known to have very high flavonoid content.

Aim of the Study.

In this study, we investigated the possibility of using TLC coupling with the MALDI-TOF-MS method and LC-MS/MS as reference tool to determine some flavonoid aglycones of *Lathyrus. pratensis* L. (herb), *Lupinus polyphyllus* L. (fruits), *Thermopsis lanceolata* R. Br. (herb), and *Sophora japonica* L. (buds), respectively, which can inhibit the α -amylase activity.

Materials and Methods. In a local pharmacy, a dry sample of *S. japonica* (buds) was purchased. Fresh herbs of *T. lanceolata* R.Br. and *L. pratensis* L. (herb), and fruits and pods of *L. polyphyllus* L. were collected from the Botanical Garden of Sechenov First Moscow State Medical University, Moscow, Russia in July 2021. The components with anti-amylase activity were isolated by TLC-bioautography assay. Alcohol extracts from plants were separated by TLC, mobile phase hexane:ethyl acetate:glacial acetic acid (20:9:1). The plate with separated extracts was then saturated sequentially with amylase, starch and iodine solutions and incubated at 37°C in a humid medium after each step. Areas coloured blue were observed on the plate, which corresponded to substances with anti-amylase activity. Identification of bioactive substances was carried out by LC-MS and MALDI-TOF-MS methods. MALDI-TOF-MS measurements were performed using a Bruker autoflex maX (Bruker Daltonik GmbH, Bremen, Germany) with a MALDI laser source (355 nm, 2000 Hz) and a TOF detector. The LC-MS method was used to confirm the MALDI results. The study was performed using a Nexera LC-30 chromatographic system (Shimadzu, Japan), with SPD-M20A diode-matrix detector (Shimadzu, Japan) and LCMS-8040 triple mass spectrometer (Shimadzu, Japan). Agilent Zorbax Eclipse XDB-C18 chromatographic column (2.1*150 mm; 5 μ m) at 40°C.

Results and Discussion. On TLC plates after bioautographic examination, distinct blue spots with an R_f of about 0.2 and R_f of about 0.6 were observed. The flavonoids genistein, apigenin, luteolin, kaempferol, formononetin were identified using MALDI-TOF-MS method. On the mass spectrometer ions with m/z 269.45 which corresponds to formononetin; m/z 271.24 which corresponds to genistein / apigenin, and m/z 286.44 which corresponds to luteolin / kaempferol were observed. These data were also confirmed by the LC-MS method. In this study, we demonstrated that the use of MALDI-TOF-MS method and LC-MS/MS allowed the preliminary characterization of flavonoid mixtures in ethanol extracts of *L. pratensis* L. herb, *L. polyphyllus* L. fruits, *T. lanceolata* R. Br. herb, *S. japonica* L. buds. The inability of MALDI-TOF-MS to distinguish between related chemical structures, on the other hand, may be due to the mass (m/z) similarity of the flavonoids themselves.

Conclusions. The use of a combination of TLC techniques with mass spectrometry, e.g. MALDI-TOF-MS, allows the identification of compounds with a certain activity without time-consuming sample preparation. The results of the study can be used for further investigate the anti-amylase activity of the components of medicinal plants.

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FLAVONOIDS OF *LYCHNIS CHALCEDONICA* PLANTS AND THEIR CULTURES *IN VITRO*Golovatskaya Irina F.¹, Kadyrbaev Maksat K.¹, Medvedeva Julia V.¹,Boyko Ekaterina V.¹, Laptev Nikolay I.¹, Matveikina Daria A.¹¹National Research Tomsk State University, 634050, Tomsk, Lenin Ave., 36, Russian Federation

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In this work, the accumulation of flavonoids in the *Lychnis chalconica* plant and its callus and suspension cultures was studied by HPLC. The organ specificity in distribution of rutin, quercetin and dihydroquercetin (taxifolin) was established.

Introduction. The solution of the problem of raw material availability in the pharmaceutical industry is associated with the study of secondary metabolism in plants of natural populations. Among biologically active substances, plant flavonoids that perform antioxidant functions both in plants and humans are in wide demand. On the basis of flavonoids it is possible to create new highly active drugs with anti-inflammatory, anticancer, antiviral, antiparasitic or bactericidal activity [1].

Aim of study. The aim of the research was to study the composition of individual substances of flavonoid nature of *Lychnis chalconica* L. plants and cultures obtained from them *in vitro*. The possibility of using *in vitro* cell cultures instead of natural population plants in practice should be evaluated.

Materials and Methods. The content of three flavonoids: rutin (R), quercetin (Q) and dihydroquercetin (DQ, taxifolin) was analyzed by HPLC method. The biochemical composition of leaves, flowers and roots of plants cultivated on sod-podzolic soils of Tomsk region (Russia) at the flowering stage was analyzed. The similar composition of callus and suspension cultures obtained from root explants of *L. chalconica* sprouts was studied.

Results and Discussion. We found organ specificity in flavonoid metabolism. DQ was predominantly present in the roots, whereas R was present in the flowers and upper leaves. Analysis of individual flavonoids showed a decreasing trend in R in the organ series: flowers > leaves > root. The distribution of Q showed an inverse R dependence, but with a significantly lower absolute level of flavonoid. The content of DQ in leaves is two times lower ($p < 0.05$) than in flowers. However, the content of this flavonoid in the roots was many times higher ($p < 0.05$) than in the above-ground organs. In the callus culture obtained from the root of *L. chalconica* sprouts, by 28 days of cultivation, the level of DQ was also shown to be many times higher relative to its content in flowers and leaves of plants at the reproductive stage. This indicated a high continuity of root cells in the synthesis of this group of flavonoids, even when isolated from the original explant. However, the specificity of the existence of cells within the callus tissue determines the peculiarities of flavonoid metabolism. Callus culture compared with the natural organ 3-fold increased R content and 2-fold increased Q content ($p < 0.05$). During the growth activation of the suspension culture, the content of R and Q ($p < 0.05$) decreased by 14 days compared to the original callus culture. The obtained data indicate the possible involvement of flavonoids in the regulation of *in vitro* culture growth.

Conclusion:

1) The presence of flavonoids increases the importance of ecdysterone-containing *L. chalconica* for use as a medicinal raw material.

2) Callus and suspension cultures of *L. chalconica* are full-fledged substitutes of plants of natural population on the content of three studied flavonoids.

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**STUDY OF THE ANTIOXIDANT ACTIVITY
OF SEA-BUCKTHORN BERRIES DURING TREATMENT WITH LIQUID NITROGEN**

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Keywords: *sea buckthorn, liquid nitrogen, phenolic compounds, flavonoids.*

Introduction. Sea buckthorn has a wide range of biological and pharmacological activities, including antitumor properties. The antitumor activity of sea buckthorn can be attributed to antioxidant compounds, in particular phenolic compounds such as flavonoids, which protect cells from oxidative damage that can lead to genetic mutation and cancer [1]. Preservation of berries by freezing, to preserve raw materials from spoilage, often leads to a deterioration in organoleptic qualities, as well as a loss of vitamins. The advantage of modern cryogenic freezing is reduced freezing time, reduced dehydration, maximum preservation of nutrients, as well as improved texture of berries due to the appearance of small ice crystals.

Aim of the Study. In this study, we evaluated the change in the antioxidant activity of sea buckthorn berries after treatment with liquid nitrogen at different time intervals.

Materials and Methods. A study was made of extracts of sea buckthorn berries after treatment with liquid nitrogen. Treatment with liquid nitrogen was carried out with different durations. Method of infusion: weighing 2 g of chopped fruits of berries and berry puree (for an extract with a concentration of 0.1 g/cm³) are placed in flasks with a ground stopper, add 10 ml of a mixture of distilled water and aqueous ethanol 1:1), kept in a thermostat at 37°C for 2 hours [2].

Results and discussion. The greatest destruction of intercellular walls with the release of more phenolic compounds and flavonoids occurs during treatment with liquid nitrogen for 5 minutes. Further treatment with liquid nitrogen does not promote the growth of released phenolic compounds and promotes the destruction of flavonoids. Thus, the content of phenolic substances in berries after 5 minutes of treatment with liquid nitrogen is 200 mg of gallic acid / 100 g of feedstock, 10 min – 181 mg of gallic acid / 100 g of feedstock, 15 min – 176 mg of gallic acid / 100 g of feedstock, 20 min – 158 mg of gallic acid/100 g of raw material, 25 min – 138 mg of gallic acid/100 g of raw material. The amount of flavonoids according to the results of the study in berries that have passed 5 min – 124 mg catechin / 100 g of raw material, 10 min – 108 mg catechin / 100 g of raw material, 15 min – 91 mg catechin / 100 g of raw material, 20 min – 54 mg catechin / 100 g of raw materials, 25 min – 42 mg of catechin / 100 g of raw materials.

Conclusions. The study found that the amount of phenolic compounds and flavonoids found in sea buckthorn berries after treatment with liquid nitrogen for 5 minutes. Prolonged processing contributes to the destruction of a large amount of antioxidants, followed by a decrease in antioxidant activity.

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ON THE MEDICINAL SPECIES OF THE *LEGUMINOSAE* IN THE FLORA OF THE CAUCASUS

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Introduction. Currently, one of the main themes of research in pharmacognosy and pharmaceutical botany is the search of the new species of medicinal plants in the Russian flora. One of the hotspots of biodiversity of our country is the Caucasus region, on the territory of which a large number of medicinal and potentially medicinal plant species are grow.

Aim of the Study. The aim of the study is to analyze medical representatives of the *Leguminosae* in the flora of the Caucasus.

Materials and Methods. During the revision of the family *Leguminosae* for the publication of the Caucasian Flora Conspectus, an analysis of the medicinal flora of this family, based on the study of herbarium samples and observations, was carried out.

Results and Discussion. More than 4,000 species grow on the Russian Caucasus region, of which about 700 belong to the legume family. Among them, there are both endemics of the flora of the Caucasus and species growing in the territory of Eastern Europe, the Mediterranean, Western and Central Asia. The medicinal flora of the family is about 100 species. More than 10 of them are officinal in Russia and other countries of Europe and Asia. These include such well-known medicinal plants as *Glycyrrhiza glabra*, *Astragalus dasyanthus*, *Genista tinctoria*, *Galega officinalis*. Other species are used in folk and traditional medicine by the people of the Caucasus and other regions, and can serve as objects of ethnobotanical research. This group includes species of wild flora and introduced and adventices species. The main areas of growth of medicinal species are the floristic regions of the North (Azov-Kuban, Belo-Labinsky and Tuapse-Adler), Central (Malkinsky and Verkhnetersky regions) and the Eastern Caucasus (Verkhnesulaksky and Manas-Samursky regions). Most of this species belong to the tribes *Genisteae*, *Sophoreae*, *Galegeae*, *Hedysareae*, and *Viciae*. They can be used as sources of quinolizidine alkaloids, flavones, isoflavonoids, triterpene saponins, coumarins.

Conclusions. A large number of potentially valuable medicinal plants from the legume family grow in the Caucasus. The most perspectives for study are the species of the genera *Genista*, *Laburnum*, *Onobrychis*, *Sophora*. It is also important to study cultivated species and introduce promising medicinal species of wild-grown flora into culture.

EVIDENTIAL HOMEOPATHY ON THE EXAMPLE OF MASTODYNONE

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Annotation. The advantages of combining the principles of allopathic and homeopathic methods of treatment that underlie the preparation “Mastodinon” are described, due to which it can be considered as an allopathic herbal preparation, and not a homeopathic remedy.

Keywords: *Mastodinone, herbal medicine, Vitex agnus castus L., Cyclamen purpurascens Miller., Iris versicolor L., Caulophyllum thalictroides (L.) Michx., Strychnos ignatii P.J. Bergiu, Lilium lancifolium Thunb.*

Introduction. Modern methods of treating various diseases are subject to modernization over time. Previously, there were several areas in medicine that were considered mutually exclusive, these are homeopathy and allopathy. At present, there is a tendency to combine the basic principles of these areas, which can be clearly illustrated by the example of the herbal drug «Mastodinon».

Aim of study. Theoretical substantiation of the effectiveness of the homeopathic preparation “Mastodinon”.

Materials and methods. The systematization of available scientific data regarding the characteristics of the chemical composition and the pharmacological effects of each component of the drug formed the basis of this work.

Results and discussion. As a result of an in-depth study of scientific publications, an analysis of the effects on the body of both each individual component and in the complex as part of the Mastodinon preparation, based on a large number of preclinical and clinical studies at various levels, the safety and high efficacy of the drug when used as a symptomatic a remedy that relieves the manifestations of premenstrual syndrome, fibrocystic mastopathy, various hormonal disorders that lead to the development of infertility and is used for irregular menstrual cycles. It should be noted that the homeopathic dilutions used in the preparation of the Mastodinone preparation are low potent and are not critical for determining the active ingredients in them, but the combination of the allopathic dose of *Vitex agnus castus L.* and homeopathic concentrations of extracts of other plants (*Cyclamen purpurascens Miller., Iris versicolor L., Caulophyllum thalictroides (L.) Michx., Strychnos ignatii P.J. Bergiu, Lilium lancifolium Thunb*) mutually enhances the action of each component, which reduces the chronicity of the disease and allows not only to reduce the time of patient treatment, but also to reduce the cost of pharmacotherapy.

Conclusions. The example of the drug “Mastodinon” shows the successful integration of homeopathic principles of treatment into allopathic medicine, which is confirmed by an extensive evidence base.

**TITLE: CHEMOSENSITIZATION OF CHEMORESISTANT PROSTATE CANCER BY LUPEOL:
TARGETING AR/MYC/B-CATENIN/C-FLIP AXIS****Hifzur R Siddique**Molecular Cancer Genetics & Translational Research Lab, Section of Genetics,
Department of Zoology, Aligarh Muslim University, Aligarh-202002 INDIA**E-mail:** hrsiddique@gmail.com**Keywords:** *Lupeol; Chemosensitization; AR; c-Myc, Wnt/β-catenin, c-FLIP, Adjuvant.*

Context/Purpose. Limitations of different therapies are the acquired development of resistance against therapies in the transformed cells of cancer patients, which leads to cancer recurrence and metastasis. Enzalutamide is the second-generation potent androgen receptor (AR) antagonist used against metastatic and non-metastatic prostate cancer (CaP). Unfortunately, the development of chemoresistance in the transformed cells reduces the effectiveness. Generally, MYC, β-catenin and c-FLIP play a crucial role in drug resistance. We have been working on a molecule, Lupeol, found in different fruits, vegetables, and medicinal plants to (i) chemosensitize the enzalutamide-resistant CaP cells and (ii) reduce the Enzalutamide-induced toxicity.

Methods. We performed *in silico*, *in vitro*, and *in vivo* studies. Molecular docking and MD Simulation [RMSD, RSF, SASA, Rg, Ramachandra Plot, number of H-bonds, PCA and Coulombic and Lennard-Jones] were done *in silico* studies. We used normal cells (PNT2), cancer cells 22Rv1, C4-2b, and Du145 and CD133⁺/CD44⁺ positive cells Cancer Stem Cells (CSCs) for our *in vitro* studies. We treated the cells with either Lupeol (10-50 μM), enzalutamide (1.0-10 μM), or a combination of both for cell growth, proliferation, migration, viability, and reporter genes assays. Next, we performed *in vivo* studies on Swiss albino mice for oxidative stress markers and genotoxicity assays.

Results & Discussion. Lupeol and Enzalutamide were docked with AR, β-CATENIN, c-FLIP_L, and c-MYC. The following MD simulation data showed both compounds exerting structural changes in these proteins. Finally, they significantly inhibit the transcriptional activity of all these genes, as observed by reporter gene assays. We further observed that Lupeol significantly inhibits the growth of CSCs sparing normal cells and chemosensitizes the CSCs for enzalutamide. Enzalutamide therapy often fails in patients with castration-resistant prostate cancer (CRPC) patients. Lupeol co-treatment significantly sensitized CRPC cells for Enzalutamide treatment. Lupeol was observed to act as a double-edged sword for AR. Lupeol reduced the transcriptional activation, mRNA level and protein level of AR in drug-resistant cells. Lupeol also reduces the transcriptional activities of AR, β-CATENIN, c-FLIP_L, and c-MYC.

Conclusion. Lupeol alone or as an adjuvant to current therapies could be developed as an agent to treat a subtype of human cancers exhibiting constitutive activation of AR, β-CATENIN, c-FLIP_L, and c-MYC. Our analysis also indicates that Lupeol can be used as an adjuvant for reducing the toxic effects and enhancing the effectiveness of Enzalutamide.

ВЛИЯНИЕ СМЕСИ СОКОВ БРОККОЛИ И ДАЙКОНА НА МОЛЕКУЛЯРНЫЕ МАРКЁРЫ СД2 И ОЖИРЕНИЯ

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Keywords: *C-пептид, лептин, адипонектин, ожирение.*

Актуальность. Лептин является гормоном жировой ткани, регулирующим аппетит и обмен веществ. Дефицит лептина характеризуется снижением чувства сытости, что приводит к чрезмерному потреблению питательных веществ и развитию ожирения. Адипонектин также относится к гормонам жировой ткани, продукция и концентрация в крови которого снижается при ожирении.

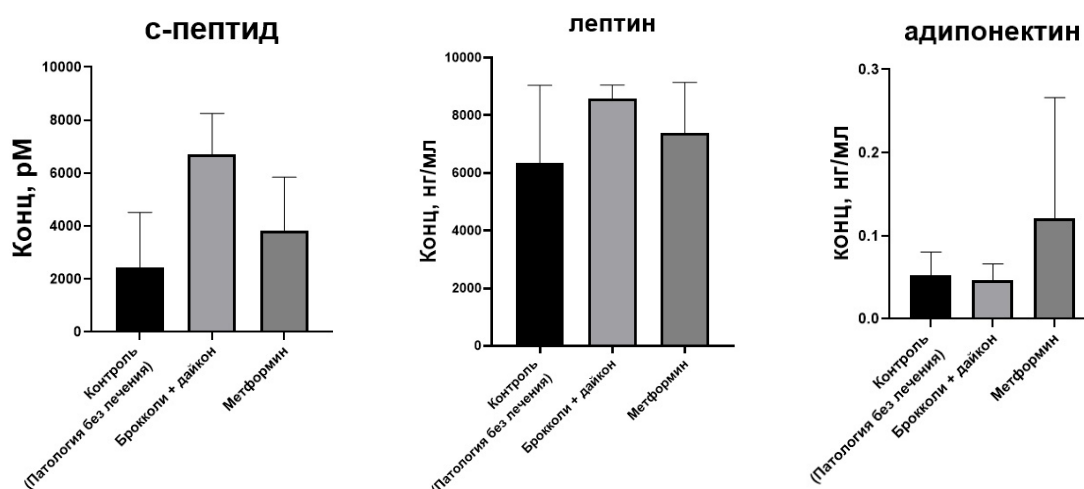
Цель. Оценить влияние смеси соков дайкона и брокколи на уровни лептина, адипонектина и С-пептида.

Материалы и методы. Для оценки влияния испытуемого образца использовали чёрных мышей линии C57BL/Ks-db+/+m. Данную тест систему можно охарактеризовать следующим образом: окрас шерсти чёрный; инбридинг Fn+8. Генотип a, db+/+m, животные несут рецессивный ген диабета db (8-я группа сцепления, 4-ая хромосома). Ген db в гомозиготном состоянии вызывает диабет, сопровождается аномальным ожирением. Гликемия очень высокая, уровень утилизации глюкозы снижен, дефицита инсулина нет, самцы и самки бесплодны, ген m- misty- рецессивный, осветляющий окраску маркер оппозитной хромосомы, не несущий гена db.

Схема введения

Испытуемый образец вводили внутривентрикулярно в течение 14 дней ежедневно в объёме 0,5 мл/животное. Животные контрольной группы получали воду очищенную в эквивалентных количествах. Также использовали препарат сравнения – метформин (Сиофор®) в дозе 300 мг/кг, вводили в виде суспензии.

Результаты



Через 14 дней введения были получены образцы крови из ретроорбитального синуса, центрифугированы и подвергнуты ИФА-анализу. По С-пептиду: все группы с нормальным распределением, one-way ANOVA положительный, в качестве апостериорного теста использован тест Тьюки, есть статистически значимые различия между брокколи и контролем, но нет различий между брокколи и метформинном; По лептину все с нормальным распределением, ANOVA отрицательный; По адипонектину: дисперсионный анализ показывает отсутствие значимых отличий между группами.

**ASSESSMENT OF POTENTIAL GENOTOXICITY OF THE PHLOROTANNIN PREPARATIONS
DEMONSTRATING HIGH ANTIBIOTIC AND ANTIFUNGAL ACTIVITIES****Islamova Renata¹, Zamyatkina Elizaveta¹, Ilinykh Sofiya², Stepchenkova Elena^{1,3}, Tarakhovskaya Elena^{1,3}**¹ Saint Petersburg State University, Saint Petersburg, Russia² Saint-Petersburg State Chemical and Pharmaceutical University, Saint Petersburg, Russia³ Vavilov Institute of General Genetics, Saint Petersburg Branch, Saint Petersburg, Russia**E-mail:** renata.tag.isl@gmail.com

Introduction. Among the bioactive metabolites produced by marine organism, phlorotannins currently attract special attention due to their high antibiotic, antifungal, and cytotoxic capacities. Phlorotannins are unique phenolic metabolites of brown algae – oligomers and polymers of phloroglucinol (1,3,5-trihydroxybenzene). Brown algae contain from 0.5 to 30% phlorotannins per dry weight. Different phlorotannin preparations are currently extensively studied from the perspective of their use in clinical pharmacy. However, these biologically active compounds are still not tested for their possible deleterious side effects, such as mutagenic activity.

Aim of study. In this study we tested antibiotic, antifungal, and mutagenic activity of three phlorotannin preparations in order to assess their perspectives for applied relevance in medicine.

Materials and methods. Phlorotannins were isolated from thalli of three brown algae (*Fucus serratus*, *Ectocarpus siliculosus*, and *Desmarestia aculeata*). Antibacterial and antifungal effects were estimated as minimum inhibitory concentrations (MIC) of phlorotannin preparations against two model objects: Gram-negative bacteria *Escherichia coli* strain KA796 and ascomycete yeast *Saccharomyces cerevisiae* haploid strain LAN201-ura3Δ. Mutagenic activity of the extracts was assessed in the Ames test using three tester strains of *Salmonella typhimurium* (TA97, TA98, and TA100). Rat liver extract was used for the metabolic activation of the potential promutagens.

Results and discussion. All three tested phlorotannin preparations showed considerable antibacterial and antifungal activity. Phlorotannins of *D. aculeata* were the most toxic with MIC values 5 and 4 µg/ml for *E. coli* and *S. cerevisiae*, correspondingly. MIC values of *F. serratus* extracts were 20 µg/ml for *E. coli* and 10 µg/ml for *S. cerevisiae*. Phlorotannin preparations isolated from *E. siliculosus* showed MIC 25 µg/ml for both test-objects. These MIC values are similar to those of widely used antibiotics and fungicides, such as tetracycline, ampicillin, fluconazole and amphotericin B. Phlorotannins of *D. aculeata* showed no mutagenic effects in the both variants of the Ames test (with and without metabolic activation). Meanwhile the phlorotannin preparations of *E. siliculosus* and *F. serratus* demonstrated slight mutagenic activity (fold change 1.3-1.4, P<0.05) compared to the negative control after metabolic activation for TA100 strain (base pair substitutions), and preparations of *F. serratus* also showed considerable mutagenic activity (fold change 2.3, P<0.05) without metabolic activation for TA97 strain (frameshift mutations).

Conclusions. Phlorotannin preparations of *D. aculeata* featured the maximal antibacterial and antifungal activities and did not demonstrate significant genotoxicity in the Ames test. Thus, these preparations may be regarded as the most perspective for further use in clinical pharmacy.

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DEGRADATION KINETICS OF DYE OBTAINED FROM POKEWEED BERRIES

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Keywords: *Phytolacca americana* berry, betacyanin, degradation, temperature, ethanol, kinetics.

Introduction. Numerous studies gradually note the development of allergic reactions of varying degrees, disruption of the natural functioning of tissues and organs, as well as the increase of severe chronic diseases associated with the use of products containing synthetic dyes. In this regards, betacyanin pigment from berries of pokeweed () could be a good candidate for natural dyes. However, the stability and acceptability of natural dyes colour were dependent on various physical and chemical factors as well as on profile of origin source. For several years, we have been carrying out research for obtaining of betacyanins from pokeweed berries and, according to developed technological scheme for extraction and purification, we obtained a red-violet dye in the form of a powder.

Aim of study was to investigation of degradation processes of natural dye obtained from pokeweed berries depending on the solvent and storage temperature.

Material and methods. The dye in aqueous and water-ethanol solutions of various concentration (20, 30, and 40%) were studied under controlled conditions at +4, 20, 30 and 40°C. Quantification of basic colour compounds betacyanins was done at 540 nm by UV-visible spectrophotometer. The concentration of betacyanins was calculated using the molar extinction coefficient ($\epsilon = 60\ 000\ \text{L mol}^{-1}\text{cm}^{-1}$) and molecular weight ($550\ \text{g mol}^{-1}$). In diapason of betacyanin concentrations from 0 to 250 mg L⁻¹ the dependence of absorption on concentration is linear with high approximation ($R=0.9998$). Since the degradation rate of betacyanins strongly depends on the degree of dilution (maximum in the most diluted sample), solutions with the same dye concentration (1.0-1.2 g L⁻¹) were used in the experiments.

Results and discussion. The degradation of pokeweed dye in water was described by a first order reaction. The reaction rate increased significantly with temperature rising. Aqueous solutions of dyes at temperature of $+4\pm 2^\circ\text{C}$ lost up to 50% of initial content during 38-40 days, but at a temperature of $+40\pm 1^\circ\text{C}$ only within 46-50 hours of storage. Using the constants of reaction rate and the Arrhenius equation, the activation energy of tested dye was calculated. Therefore, the activation energy of degradation reaction of the dye aqueous solution was $63.8\ \text{kJ mol}^{-1}$. The kinetics of dye degradation in ethanol solutions had an exponential dependence on storage time. The reaction rate increased not only with an increase in temperature, but also with a rise in the concentration of alcohol. Thus, the loss of 50% betacyanins concentration at $+40\pm 1^\circ\text{C}$ was done during 34-36, 24-26, 18-20 hours, respectively in 20, 30, 40% ethanol solutions. Such degradation kinetics of ethanol solutions can be explained by some chemical interactions between ethanol and betacyanins. The higher reaction of degradation may be due to the nucleophilic attack by ethanol on the aldimine bond of betacyanins, which leads to their decarboxylation and isomerization with colour change. It should be noted that the shelf life of pokeweed dye in powder form at room temperature is more than two years, and films based of carboxymethylcellulose sodium salt containing 0.2% of dye were also stable.

Conclusions. The degradation kinetics of natural dyes obtained from pokeweed berries substantially depends on both the storage temperature and the solvent. Pokeweed dye can be recommended for use in ethanol-free food and cosmetic products as well as in products with low water activity.

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MYCOTOXINS CONTENT IN LICORICE ROOTS

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Keywords: *mycotoxins, ochratoxin A, UHPLC-MS/MS, licorice roots.*

Introduction. Mycotoxins are secondary metabolites of microscopic mold fungi. They carry a serious threat to human and animal health. Mycotoxins can affect many organs and organ systems, especially the liver, the kidneys, the nervous and immune systems of the body. Moreover, some mycotoxins have been classified as carcinogens of class I and II according to the International Agency for Research on Cancer (IARC) [1]. Currently, the maximum permitted concentrations of mycotoxins in the Russian Federation are regulated only in food products under the Technical Regulation of the Customs Union 021 “On food safety”. Medicinal plant raw materials are not standardized in the Russian Federation, consequently they may threaten human health. Therefore, it is important to assess the contamination of plant raw materials with mycotoxins [2].

Aim of study is to conduct a research on contamination of plant raw materials of licorice roots with mycotoxins of the fungi species *Aspergillus*, *Penicillium*, *Fusarium* и *Alternaria*.

Materials and methods. The research was conducted by the method of ultra high-performance liquid chromatography-MS/MS (UHPLC-MS/MS) in 7 samples of plant raw materials of licorice roots. The content of mycotoxins of the following groups was identified: deoxynivalenol, aflatoxins, ochratoxin A, zearalenone, T-2 toxin, fumonisins, sterigmatocystin, HT-2 toxin, diacetoxiskirpenol, neosolaniol, citreoviridine, mycophenolic acid, citrinin, tentoxin, alternariol and its methyl ester.

Results and discussion. In the examined samples the following mycotoxins were found: alternariol methyl ether in 14% of samples in the amount of 15.12 µg/kg, alternariol in 14% of samples in the amount of 648.98 µg/kg, citrinin in 14% of samples in the amount of 2.59 µg/kg, deoxynivalenol (DON) in 29% in the range from 66.85 to 68.51 µg/kg, fumonisin B1 in the amount of 120.17 µg/kg, fumonisin B2 (FB2) in 29% of samples in the range from 31.80 to 34.69 µg/kg, mycophenolic acid (MPA) in 86% of samples in the range from 1.00 to 44.34 µg/kg, neosolaniol in 14% of samples in an amount of 2.54 µg/kg, ochratoxin A (OTA) in 14% of samples in the amount of 24.01 µg/kg, ochratoxin B in 14% of samples in the amount of 4.87 µg/kg, T-2 toxin in 14% of samples in the amount of 4.86 micrograms, tentoxin (TE) in 29% of samples in the range from 13.04 to 18.14 µg/kg, zearalenone in 14% of samples in the amount of 43.97 µg/kg. More than 50% of the samples were contaminated with more than one mycotoxin (DON, FB2, MPA, TE). The level of ochratoxin A exceeded the maximum permitted level established by the European Pharmacopoeia (20 µg/kg) in 14% of licorice root sample.

Conclusions. The data obtained show high frequency of contamination of licorice roots with mycotoxins, especially with ochratoxin A and mycophenolic acid. Consumption of contaminated licorice roots raw materials may lead to a threat to human and animal health. Therefore, the development of standards of content and control of mycotoxins in medicinal plant raw materials is required.

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TECHNOLOGY OF OBTAINING LIQUID EXTRACT FROM *ARTEMISIA SEROTINA* BUNGE**Kadyrbai A., Sakipova Z.B., Ibragimova L.N., Akhmetova K.T., Bekenova Zh.A., Kazim A.Yu.**¹Department of Engineering Disciplines and Good Practices, School of Pharmacy,
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Introduction. At the School of Pharmacy of Kazakh National Medical University named after S.D. Asfendiyarov studies of an endemic plant species *Artemisia serotina* Bunge of the genus *Artemisia* of the *Asteraceae* family [1, 2] are held to justify its use in medicine, including the pharmaceutical industry. A technology for the collection and processing of medicinal plant raw materials has been developed, its standardization has been carried out, also its pharmaceutical and technological parameters and stability have been determined.

Aim of the Study. Development of an optimal technology for obtaining liquid extract from *Artemisia serotina* Bunge herb of pharmacopoeial quality.

Materials and Methods. As the object of study were used the raw materials of *Artemisia serotina* Bunge herb, harvested in the period from August to September 2022 in accordance with the principles of GACP. The method of obtaining the extract is experimentally substantiated – maceration with the use of ultrasonic treatment.

Three parameters were studied in the experiment: ultrasound frequency from 25 to 40 Hz, duration of ultrasonic exposure from 2 to 10 minutes, temperature from 25 to 60 °C. 1.0 g of raw material was loaded into a flask with a volume of 100 ml, brought to the mark with an extractant ethyl alcohol 50%. The raw materials were infused for 4 hours, then they were exposed to ultrasound at 25 and 40 Hz. The experiment was carried out at least three times with each frequency in the time regulation: 2 min, 4 min, 6 min, 8 min, 10 min and temperature from 25 °C to 60 °C.

Statistical processing of the results was carried out using the Minitab program.

Results and Discussion. According to the principles of Quality by Design, the optimal parameters in the technology for obtaining a liquid extract are determined: the extraction method is maceration with ultrasonic treatment, the frequency of ultrasound is (25–40) Hz, the time of ultrasonic treatment is (2–4) min, the multiplicity of ultrasonic treatment is at least 3 times, temperature – (40 – 50) ° C.

Thus, the optimal technological parameters have been determined, a technological scheme has been developed, control points of the technological process have been determined, and a quality specification for the finished product and laboratory regulations have been developed for obtaining a liquid extract from *Artemisia serotina*.

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THE BUILDING OF PHYTOCHEMICAL AND RESOURCE MAPS
AS PART OF THE MEDICINAL FLORA STUDY

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Keywords: *phytochemical maps, resources, GIS, medicinal flora.*

Introduction. Pharmacognostic scientists have been using paper atlases of habitats and resources for many years when planning expedition routes, as well as for studying areas where medicinal plants grow. It is important to emphasize that updating the paper cartographic material is possible only by republishing the atlas, which takes time and financial resources. Currently, in the field of forest management, geography, botany, geographical information systems such as ArcView, ArcGIS, QGIS, etc. are widely used to build electronic thematic maps. In our opinion, the creation of multifunctional electronic cartographic material can also find its application in the research of medicinal plant resources and will become a convenient tool for pharmacognostics, harvesters of medicinal plant raw materials and manufacturers of herbal medicines.

Aim of the Study. In this article, we describe the possibilities of assessing the state and convenient analysis of medicinal flora by using GIS on the example of the Perm Region.

Materials and Methods. Expeditionary resource studies were conducted in the summer of 2022 in the Perm Region districts. The coordinates of the medicinal plants growth were determined by the Garmin eTrex Vista C navigator. Resource indicators were determined according to the generally accepted method of determining stocks of medicinal plants. Phytochemical quality indicators were determined according to the requirements in the 14th edition of the Russian Federation's State Pharmacopoeia.

The Microsoft Excel program was used to process information and form a database. Electronic thematic GIS maps were built in the ArcView program. The topographic basis for the construction of electronic maps was provided by the GIS Center of the Perm State University.

Results and discussion. As part of the work, own expeditionary research was carried out on the territory of 13 districts of the Perm Region. The growth places were determined and the raw materials of the most common medicinal plants were studied, including *Hyperici herba*, *Achillea millefolium*, *Artemisia absinthium*, *Leonurus quinquelobatus*, *Tanacetum vulgare*, *Origanum vulgare*. For each studied species, the main resource indicators are calculated: the thicket area, the density of raw materials, biological reserve of raw materials, the possible annual harvesting volume. Then the main quality indicators of medicinal plant raw materials and the biologically active substances amount were determined, the requirements for which are described in private pharmacopoeia articles. It is important to note that the degree of therapeutic effect of a medicinal plant depends on the quality of raw materials and the amount of biologically active substances.

As a result, a database was formed, which was loaded into the ArcView 3.2a program. It is possible to update information by adding or changing data in an electronic information database. Data analysis for the construction of electronic maps was carried out automatically. So, thematic phytochemical maps and distribution maps were built for all the studied species in the studied districts of the Perm Region. Electronic maps are a multifunctional tool, since it is possible to interact with individual objects on the map (choosing a specific medicinal plant, area, resource characteristics or phytochemical characteristics), viewing indicators for each type of medicinal plant raw materials by localization. The use of GIS will allow you to quickly, visually and effectively assess the current state of medicinal flora and find the most rational places for harvesting medicinal plant raw materials of appropriate quality. This material can become a convenient tool for pharmacognosts within the framework of the issue of rational nature management and monitoring of available domestic plant resources. Thematic maps will also be useful for harvesters of medicinal plant raw materials and manufacturers of effective medicines and biologically active additives on a plant basis. Research continues.

**CONTINUUM OF PHYTOPREPARATION DEVELOPMENT:
EXPERIENCE OF LABORATORY-INDUSTRIAL TRANSFER**

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Introduction. The analysis of the state register of medicines showed the presence of antitumor drugs of plant origin on the EAEU market, which are effective official medicines. However, most of them are produced abroad. The development of domestic drugs is relevant due to the need for import substitution. Publications of Russian researchers in the field of the use of Avran medicinal extract in oncology have shown its potential effectiveness, which is why the transfer of its production to industrial pharmacy and the solution of issues of subsequent introduction to the EAEU market is relevant. The extract developed earlier by the laboratory method needed to transfer the development to industrial production for the manufacture of batches suitable for clinical research on it.

Aim of the Study. Assessment of pharmacoeconomic basis of laboratory-industrial transfer and continuum of phytopreparation development.

Materials and methods. The development of pilot production regulations was carried out, pilot batches were created, methods of quality control of raw materials, active pharmaceutical substance (AFS) and finished dosage form (GLF) were developed, they were tested in laboratory conditions, and then – in the GMP–certified laboratory of the certified manufacturer JSC “Farmcenter VILAR”, the Russian pharmaceutical site.

Results and Discussion. Employees of the Saratov State Medical University named after V.I. The Razumovsky Ministry of Health of Russia assessed the resources of Avran medicinal raw materials in the Saratov region, organized the processes of collection, procurement and storage of raw materials according to pharmacopoeia requirements, developed pilot industrial regulations (OPR) for the procurement of raw materials and technical specifications. Several batches of raw materials were collected, and their compliance with regulatory requirements was assessed. All batches meet the requirements and can be used for the production of AFS. Methods of quantitative determination of active substances in raw materials, AFS and GLF have been developed and validated. The results obtained formed the basis of regulatory quality documents for Avran medicinal herb, AFS and AFS, as well as GLF and GLF, which in the future will become an integral part of the registration dossier, and are necessary for the continuation of the development of the drug. The university has completed the development of the AFS “Avrana medicinal extract dry” and “Avrana medicinal extract thick”, prepared and approved laboratory, and then – pilot-industrial regulations for their production through the transfer of production technology of two AFS in industrial conditions (GMP), three series of AFS, dry extract of Avrana medicinal, passed quality control in accordance with the ODA (satisfactory). All AFS series can be used to create GLF. The composition of several GLF has been developed in laboratory conditions – capsules of 330 mg and tablets of 125 mg, 250 mg, the transfer of GLF production technology to industrial conditions (GMP) has been completed, a production ERP has been prepared and approved and three series of GLF have been developed in quantities sufficient for a Phase I clinical trial scheduled for 2023 year.

Conclusion. For the first time in the Saratov State Medical University named after V.I. Razumovsky, a package of documents was created to submit for examination to the Ethical Committee of the Ministry of Health of the Russian Federation according to the requirements of the regulator to obtain approval for a phase I clinical trial, 6 pharmaceutical products were created, 8 draft regulatory documentation for approval by the regulator (the Ministry of Health of the Russian Federation, the Pharmacopoeia Committee), 6 approved regulatory documents on pharmaceutical products and technologies for its production. A transfer to the industrial site was carried out.

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SESQUITERPENE LACTONES OF THE SIBERIAN POPULATION OF *CENTAUREA SCABIOSA* L. AS A PROMISING DRUGS FOR THE OPISTHORCHIASIS TREATMENT**Kaminskii I.¹, Ivanov V.¹, Kadyrova T.¹**¹Federal State Budgetary Educational Institution of Higher Education "Siberian State Medical University"
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Introduction. Opisthorchiasis is a challenge for the regions of the Russian Federation endemic to this helminthiasis (Novosibirsk, Tomsk, Tyumen, Kemerovo regions, Komi-Permyatsky, Khanty-Mansiysk, Yamalo-Nenets Autonomous Districts). *Centaurea scabiosa* L. has long been used in folk medicine as an antiopisthorchiasis agent. Previously, it was shown that the antiopisthorchiasis properties of this plant are associated with the presence of sesquiterpene lactones in its composition [1].

Aim of the Study. In this study, we evaluated the anthelmintic action against *Opisthorchis felineus* and the mechanism of specific activity of the sesquiterpene lactone grosshemin isolated from the Siberian population of *Centaurea scabiosa* L.

Materials and Methods. Studies of antiopisthorchiasis activity in vitro were carried out on viable *Opisthorchis felineus* marites isolated from the organs of the hepatobiliary system of infected experimental animals (golden hamsters). Incubation of isolated *Opisthorchis felineus* marita with various concentrations of grosshemin was carried out in RPMI 1640 medium with the addition of 50 µg/ml benzylpenicillin sodium salt solution, 50 u/ml streptomycin solution, 80 µg/ml hemin solution at 37 °C in an atmosphere of carbon dioxide (5%). The viability of *Opisthorchis felineus* maritas was assessed after 24, 48, and 72 hours of exposure to grosshemin by the magnitude of their motor activity, assessed visually under a microscope with a twentyfold magnification. The study of the mechanism of the specific activity of grosshemin was carried out outside the body, in a culture medium containing isolated *Opisthorchis felineus* marites, as well as on a cell-free model (protein lysate or maintenance medium). Effective doses of grosshemin were determined as an indicator of IC50. Enzymes of the glutathione-S-transferase system were chosen as a target for studying the mechanism of action [2].

Results and discussion. As a result of in vitro experiments, it was shown that after 24 hours of incubation at doses of 25, 50, and 100 µg/ml, grosshemin caused 100% death of *Opisthorchis felineus* maritis. At a concentration of 20 µg/ml, grosshemin resulted in the death of 95% of *Opisthorchis felineus* marita. At a dose of 10 µg/ml, grosshemin reduced the locomotor activity of 5% marit *Opisthorchis felineus*, and at a dose of 15 µg/ml, it had a more pronounced reducing effect on locomotor activity (60% marita *Opisthorchis felineus*). It has been established that grosshemin inhibits the activity of glutathione-S-transferase sigma in the incubation medium and in the helminth lysate. The calculated parameter of the dose-response inhibition curve IC50 is 375 µM, which corresponds to the IC99 indicator of the anthelmintic effect of grosshemin. These data suggest that grosshemin, isolated from the Siberian population of *Centaurea scabiosa* L., have anti-opisthorchiasis activity against *Opisthorchis felineus*, which confirms the data of traditional medicine. Thus, the sesquiterpene lactone grosshemin is a promising drug candidate for the creation of a new drug for the treatment of opisthorchiasis caused by *Opisthorchis felineus*.

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**EXAMINING THE CONTEMPORARY AND TRADITIONAL UTILIZATION OF MEDICINAL PLANTS
IN INDIA AND RUSSIA: AN INVESTIGATION INTO THE HERBAL CONNECTION**

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Introduction. This study examines the historical and current use of medicinal plants in Russia and India, given their long-standing traditions in using such plants for healing purposes. With the criticism of conventional medicine in light of recent scientific advancements, this research also explores their herbal connections to gain a better understanding of the use of therapeutic plants.

Aims of study. This study aims to investigate the use of medicinal plants in India and Russia, identifying similarities and differences. It also evaluates scientific evidence supporting their use in modern medicine.

Materials and Methods. A literature review using PubMed and Google Scholar focused on «medicinal plants,» «herbal medicine,» «India,» and «Russia.» Only English peer-reviewed articles were included. Patterns in the use of medicinal plants in these countries were identified through analysis of the selected articles.

Results and Discussion. India and Russia have a history of using medicinal plants for health conditions. The plants used vary due to differences in climate, geography, and cultural practices. Ayurveda in India uses herbs like turmeric, ginger, and holy basil [1]. While traditional Russian medicine uses birch, chamomile, and St. John's Wort[2]. However, both use adaptogenic herbs like ashwagandha and rhodiola[1,2]. Suggesting potential for collaboration in modern medicine.

Conclusion. This study emphasizes the traditional use of medicinal plants in India and Russia. Despite limited scientific evidence, their historical use suggests potential therapeutic properties. Collaboration opportunities exist between the two countries for exploring medicinal plants in modern medicine.

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COUMARINS OF PLANTS *IN VIVO* AND CELL CULTURES *IN VITRO*Khandy Maria^{1,2}, Grigorchuk Valeria², Gorpenchenko Tatiana^{1,2}¹Far Eastern Federal University, 690922, Russian Island

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Introduction. Coumarins are a large group of secondary metabolites that have antispasmodic, sedative, diuretic, anthelmintic, analgesic, antimicrobial, antitumor and other properties. Rare groups of coumarins include furanocoumarins and pyranocoumarins, which, depending on the position of the third ring (furan or pyran, respectively), are angular and linear form. Depending on the modification of the structure, there is a change or expansion of biologically active properties. *Phlojodicarpus sibiricus*, an endemic of the Far East, was previously included in the State Register of Medicines of the Russian Federation as a source of pyranocoumarins. Now *P. sibiricus* is an endangered plant. The related species *P. villosus* may be a source of pyranocoumarins. However, its qualitative composition of secondary metabolites has been little studied.

Aim of study. Study of the individual groups composition of coumarins of *P. sibiricus*, *P. villosus* and obtaining cell cultures of these plants.

Materials and Methods. Objects: *P. sibiricus*, *P. villosus*. The air-dried and powdered samples were extracted 80% v/v methanol. Reversed-phase high-performance liquid chromatography with diode array detection and electrospray ionization high-resolution mass spectrometry method (HPLC-MS) was applied for the coumarin determination. The callus and suspension obtained on Murashige-Skoog medium with different combinations of hormones. The cell culture carried out at a temperature of 26 °C and a humidity of 70 ± 5%.

Results and Discussion. The results of HPLC-MS analysis showed that *P. sibiricus* and *P. villosus* differ significantly in coumarin composition. *P. sibiricus* is dominated by pyranocoumarins, while *P. villosus* is dominated by furanocoumarins. According to fragments of standards (visnadin and peucenin), only angular forms of coumarins were found in both species. The data are consistent with literature [1; 2]. In addition, ostenol, a precursor of angular pyrano- and furanocoumarins, was found in both species. Cell cultures of *P. sibiricus* and *P. villosus* were obtained on Murashige-Skoog medium. *P. sibiricus* and *P. villosus* cell cultures were dominated by polar compounds related to phenolic derivatives. Most of the compounds found in cells *in vitro* (with the exception of kellecton hexoside) are not found in intact plants. It should be noted that prenylated coumarins (visnadin, dihydrosamidin, and other esters of kellectone) were identified only in primary cultures of *P. sibiricus* of hypocotyl origin. They were not found in long-term cultures of *P. sibiricus* cells. The results are concordant with data known in the literature on changes in secondary metabolism in cell cultures as compared with intact plants.

Conclusions. *P. villosus* cannot be a source of pyranocoumarins, because their content in the plant is critically low. While the plant contains a large amount of furanocoumarins, which have antibacterial properties. Furanocoumarins and pyranocoumarins share a common precursor. However, the switching of directions in the biosynthesis of coumarins of different groups has not been studied. For this, in the future, the obtained cell cultures can be used.

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ANALYSIS OF THE CREAM MARKET IN THE EURASIAN ECONOMIC UNION TERRITORY

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Introduction. In the Republic of Kazakhstan (RK) and the Eurasian Economic Union (EAEU) countries, there are medicinal creams (MC) classified as medicinal products (MP) and cosmetic creams. Different requirements are imposed on the quality of these products. The perfume-cosmetic and pharmaceutical industry in the Eurasian Economic Union (EAEU) lacks statistical analysis, especially regarding specific products such as creams. Market analysis of creams can be a useful tool for developing and evaluating marketing strategies for manufacturers and distributors.

Aim of the Study. To analyze the market of medicinal creams and cosmetic creams registered in the territory of the EAEU.

Materials and Methods. For the following databases were used for market analysis: State Registers of medicinal products (MP) registered in the EAEU countries (Kazakhstan, Russian Federation (RF), Republic of Belarus (RB), Republic of Armenia (RA), Kyrgyz Republic (KR)), Unified Register of certificates of state registration registered in the territory of the EAEU.

Results and Discussion. The analysis of the pharmaceutical market of the EAEU countries in terms of medicinal and cosmetic creams showed that the largest number of creams are registered in Russia (207 products), followed by Kazakhstan (106 products), Belarus (70 products), Armenia (57 products) and Kyrgyzstan (22 products). According to the registry of Kazakhstan, the share of registered creams from the total number of drugs is 1.4%. Medicinal and cosmetic products are registered by 23 countries, of which 6.6% are domestic and 93.4% are foreign. The analysis of the market showed that among the registered foreign drugs, the top three producing countries are as follows: India – 23.6%, Germany – 9.4%, and Italy – 8.5%. Domestic creams are produced by 2 manufacturers: Pharmacia 2010 (1 MP) and Nobel Almaty Pharmaceutical Factory (6 MP). The registered medicinal products in Kazakhstan are intended for the treatment and prevention of hemorrhoids and anal fissures (3.8%), skin diseases (81.1%), diseases of the urogenital organs and sex hormones (5.7%), joint and muscle pain (7.5%), nervous system, to reduce pain sensitivity (1.9%). At the same time, domestic manufacturers only produce medicinal preparations in the form of creams for the treatment of skin diseases, accounting for 8.1%. According to the “Unified Register of Government Registration Certificates” of the Eurasian Economic Commission (EEC), the share of pharmaceuticals for human use is 17.7%, of which the share of clinical drugs is 31% (35797 items), with the status of “signed and in force” being 27.7% (32162 items). We conducted an analysis of clinical drugs by country of registration, which showed that 29543 items (82.5%) are registered in Russia, 3286 items (9.2%) in Belarus, 1676 items (4.7%) in Kazakhstan, 1089 items (3%) in Kyrgyzstan, and 203 items (0.6%) in Armenia. Among the domestic producers of registered KK in the EAEU registry, three manufacturing companies were identified: TOO “Goldman and Young” (1 item), TOO “Bioton” (10 items), and TOO “Scientific-Practical Center” RE-BIOMED “(1 item). However, it should be noted that there are unregistered cosmetic creams on the domestic market from manufacturers such as Cremative, AO “Aisel Cosmetics”, Evita Complex, AO “Interpharma-K”, and others.

This analysis indicates that the cream market is almost entirely dependent on imports, and there is also uncertified production from domestic manufacturers present in the market. Based on this, domestic manufacturers of perfume-cosmetic and pharmaceutical products should pay more attention to expanding their range and opening new production sites for the production of high-quality and competitive creams.

GREEN METHODS AND BIOTECHNOLOGIES IN EXTRACTING BIOLOGICALLY ACTIVE COMPOUNDS FROM PLANT RAW SOURCES AND FOOD WASTESKovaleva E.G.¹, Aboushanab S.¹, Slesarev G.P.¹, Lubyakina P.N.^{1,2}, Kanwugu O.^{1,2}¹Ural Federal University, Yekaterinburg, Russia²Macquarie university, Sidney, Australia

Introduction. Bioactive compounds including phenolics, proteins, alkaloids, carotenoids, sugars, or lipids, among others, have been studied intensively due to their biological properties, capable of providing multiple health benefits. They can be extracted from by-products of agricultural, food, and fishing industries, such as crustaceans' shells, plants, algae, or microalgae by-products, which can correctly contribute to a circular economy based on zero waste. Nowadays environmentally friendly protocols of extracting these compounds using physical methods, non-toxic solvents and biotechnologies are the most preferable.

Aim of the Study. This study is aimed to review green extraction technologies and biotechnologies which were used by us for obtaining biologically active substances (BAS) including isoflavones, resveratrol, piperine, astaxanthin, catechins and Chlorella Growth Factor (CGF).

Materials and Methods. We used ultrasound-assisted NADES (Natural Deep Eutectic Solvents) extraction of isoflavones from soy molasses, red clover and kudzu flowers, and kudzu roots [1,2], resveratrol from *Fallopia japonica*, piperine black pepper (*Piper nigrum* Linn) [3], astaxanthin from crayfish wastes, catechins from green (Matcha, back) tea and its wastes. There were tested 16 two and three component combinations of Hydrogen Bond Acceptors (HBA) and Hydrogen Bond Donors (HBD) and molar ratios. NADES were employed in different molar material ratios of NADES to raw source in volume (mL)/weight (g). Natural astaxanthin has also been obtained through culturing *Phaffia rhodozyma* yeast in the nutrient media containing soy (sugar) molasses and waste beer yeasts as sources of carbon and vitamins and aminoacids, respectively [4], while we applied cellulolytic enzyme Cellulox-A (Sibbiopharm Ltd., Russia) for water extraction of CGF from powdered *Chlorella vulgaris* algae [5].

Results and discussion. The maximum yields of the ultrasound-assisted NADES extraction of isoflavones, resveratrol, piperine, astaxanthin, catechins were found to be at 50-60 °C during 1-4 h: (at 1:2 choline chloride/citric acid with 20% (v/v) of water)[2]; (1:1 choline chloride/citric acid with 20% (v/v) of water); (choline chloride/citric acid/1,2-propylene glycol combination (1:2:2 molar ratio) with 25% (v/v) of water)[3]; (1:2 choline chloride/ethylene glycol with 10% (v/v) of water) (1:1 choline chloride/2,5-hexandiol with 25% (v/v) of water), respectively. The treatment of *Chlorella* algae with enzymatic preparation Cellulox-A increased the growth factor index from 3.5 to 7.5 (approximately 3% increment). The extractability of growth factor was observed to depend on extraction time with the optimal extraction yield obtained at 120 min [5]. Cultivation of *P. rhodozyma* Y1654 in sugar beat molasses (SBM) resulted in as much as twice (32.8 µg g⁻¹ of dry cell weight (DCW)) the astaxanthin yield of soy molasses (SM) (12.4 µg g⁻¹ DCW)[4].

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PHARMACOGNOSTIC STUDY OF «AMARANTH TEA» HERBAL COMPOSITION

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Summary: identity characteristics and content of BAS in herbal composition based on fermented *Amaranthus hypochondriacus* L. herb and *Chamaenerion angustifolium* (L.) Scop. leaves were studied.

Keywords: *Amaranthus hypochondriacus* L., *Chamaenerion angustifolium* (L.) Scop., *Melissa officinalis* L., *Ribes nigrum* L., *Viburnum opulus* L., *herbal composition*.

Introduction. Herbal compositions containing fermented above-ground parts of willowherb and amaranth are used as tea drinks and dietary supplements – sources of flavonoids and polyphenolic compounds. Relevance and demand for herbal composition «Amaranth tea» is due not only to its taste characteristics, but also to the complex effect of its components and their synergism, this composition is supplemented by herbal sources of anthocyanins and ascorbic acid. The development of appropriate regulatory documentation is relevant.(1)

Aim of study. Study of external and microscopic features of herbal composition «Amaranth tea», determination of biologically active substances content in raw material and water extract (prepared according to package instructions).

Materials and methods. Herbal composition from: amaranth herb (*Amaranthus hypochondriacus* L.) fermented granulated, rosebay willowherb leaves (*Chamaenerion angustifolium* (L.) Scop.) fermented granulated, melissa herb (*Melissa officinalis* L.), black currant leaves (*Ribes nigrum* L.) fermented, black currant fruits, cranberry fruits (*Viburnum opulus* L.) developed and produced by LLC «SPA NIKOLSKAYA BIOFABRIKA», Republic of Belarus. Studies were carried out according to the methods of the State Pharmacopoeia of the Russian Federation XIV edition.(2)

Results and discussion. When examining the external signs of herbal composition, it was found that the method of components preparation allows their simple identification, the content of particles less than 2 mm is insignificant, microscopic signs of components are also visualized satisfactorily. For example, raffids in the leaves of willowherb are detected in 100% of micro preparations. The content of BAS in herbal composition and water extract (n=5, P=0.95, t(95,5)=2.78) was, respectively: extractives extracted by water and dry residue 34,64±1,64% and 0,164±0,003%; tannins in terms of tannin 3,63±0,12% and 0,0150±0,0003%; anthocyanins in terms of cyanidin-3-O-glucoside 0,82±0,02% and 0,0050±0,0002%; ascorbic acid 22,2±0,3 mg% and 0,415±0,001 mg%; total flavonoids in terms of luteolin-7-O-glycoside (the maximum absorption spectrum of water-alcohol extract (70% alcohol) after the reaction of complexation with aluminum chloride coincides with the maximum of luteolin-7-O-glycoside complex with aluminum chloride) 0,489±0,013% and 0,0116±0,0005%; polysaccharides 16,50±0,20% and 0,156±0,002%.

Conclusions. The results of the study of the quantitative composition of BAS demonstrate the promising use of the herbal composition «Amaranth tea» as a dietary supplement – a source of antioxidants, trace elements, polyphenols and polysaccharides.

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**EXPERIENCE IN STUDYING THE REPRODUCTIVE TOXICITY OF HERBAL MEDICINES USED
IN THE COMPLEX TREATMENT OF RESPIRATORY DISEASES**

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Introduction. Currently, a considerable amount of experience has been accumulated, indicating the possibility of a negative effect of some synthetic drugs on pregnancy and fetus, which was a deterrent for many women and forced them to replace them with safer phytochemicals and biologically active food supplements (BAS) containing herbal medicinal raw materials. However, it is well known that herbal medicines and dietary supplements are not so harmless. Currently there is a lot of data about the negative impact of medicinal plants (*Humulus lupulus* cones, *Cimicifuga racemosa* rhizomes and roots, *Salvia officinalis* leaves, *Scutellaria galericulata* roots, *Thermopsis lanceolata* aerial part) and drugs created on their basis on reproductive function (decreased fertility, spermatogenesis index, menstrual cycle disorders, etc.) and the development of offspring in the postnatal period of life (decrease in body weight and size, impaired physical development, research behavior, learning ability) of animals and humans. To prevent undesirable pregnancy outcomes, doctors need to have reliable information about the possible consequences caused by treatment with herbal medicines and to guarantee the safety of patients, therefore, the study of the reproductive toxicity of herbal preparations at the stage of preclinical studies is relevant [1].

Aim of the Study. To evaluate the possibility of toxic effects of drugs containing dry extracts of the herb *Echinacea purpurea* and *Thermopsis lanceolata* on the development of rat offspring in the ante- and postnatal periods of development and to give recommendations on the clinical use of these drugs during pregnancy.

Materials and Methods. Studies were carried out on Wistar rats, which during 19 days of pregnancy were administered into the stomach with dry extract of *Echinacea purpurea* in doses of 50, 250 and 1000 mg/kg; dry extract of *Thermopsis lanceolata* in doses of 6 and 18 mg/kg; a combined expectorant in syrup form, containing dry extract of *Thermopsis lanceolata* at doses of 3 and 9 ml/kg. Control animals received water. Assessment of the condition of the offspring was carried out in the ante- and postnatal periods of development by generally accepted methods.

Results and Discussion. It was shown that the dry extract of *Echinacea purpurea* did not exhibit embryotoxic and teratogenic properties when administered into the stomach of rats in the studied doses. The combined expectorant herbal medicine in the form of syrup as well as the dry extract of *Thermopsis* statistically significantly reduced the body weight and craniocaudal size of 20-day-old fetuses, slowed down the process of ossification of the skeleton in 44-50% of embryos ($p < 0.05$), caused a violation of vestibular function and coordination of movements in rats relative to the control, increasing the time of taking the initial position by 1,5-1,8 times ($p < 0.05$) [2,3].

Conclusion. It was concluded that pregnancy should be considered a contraindication to the prescription of drugs containing dry extract of the herb *Thermopsis lanceolata*. Dry extract of *Echinacea purpurea* may be recommended for pregnant women.

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DETERMINATION OF THE TOTAL FLAVONOID CONTENT FROM DIFFERENT MORPHOLOGICAL PARTS OF YELLOW LOOSESTRIFE (*LYSIMACHIA VULGARIS* L.)

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Abstract: This study used total flavonoid content (TFC) determination based on UV-VIS colorimetric method. Results showed TFC in extracts of stems 2020 (0,96±0,15%), flowers 2020 (2,08±0,09%), leaves 2020 (2,90±0,09%), herb 2020 (2,63±0,07%), herb 2021 (2,99± 0,06%) of yellow loosestrife.

Keywords: Total flavonoids, quantification, yellow loosestrife, *Lysimachia vulgaris* L.

Introduction. Yellow loosestrife contains flavonoids with antioxidant activities. Medicines based on substances that can utilize free radicals formed during oxidative stress are of interest as a therapy and prevention of inflammatory diseases [1-3].

Aim of the Study. In our study we evaluated the TFC of the yellow loosestrife extracts by using the spectrophotometric method.

Materials and Methods. The materials of the study were herb, leaves, flowers and stems of the yellow loosestrife, collected in the Leningrad region in 2020 and 2021. Raw material was heated with 50 ml of ethanol 75% for 30 min and filtered. 2 ml of the filtrate and 2 ml of aluminum chloride 1% were dissolved with ethanol 95% to 25 ml. Control solution was prepared without aluminum chloride 1%. Absorbance evaluation was evaluated at 411 nm after 30 min incubation. Rutin was used as the standard. The TFC (%) was estimated according to the equation:

$$x, \% = \frac{D_x \times m_{st} \times 100 \times 50 \times 25 \times 2 \times 100}{D_{st} \times m_x \times 2 \times 100 \times 25 \times (100 - w)}$$

D_x – sample optical density, D_{st} – standard optical density, m_x – sample mass (g), m_{st} – standard mass (g), w – absolute humidity (%). MicrosoftExcel 2016 software was used for statistic analysis. All experiments were repeated five times and expressed as mean (%) ± standard deviation ($p < 0.05$).

Results and Discussion. Results revealed that the TFC in extracts of stems 2020, flowers 2020, leaves 2020, herb 2020, herb 2021 were: 0,96±0,15%, 2,08±0,09%, 2,90±0,09%, 2,63±0,07%, 2,99± 0,06% respectively. The highest amount of flavonoids was found in the leaves. More TFC was noted in the herb harvested in 2021 compared to the 2020 herb samples (Fig).

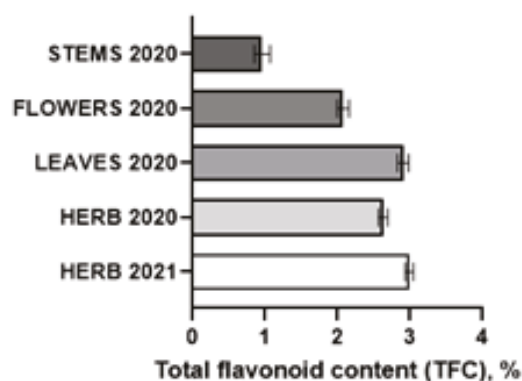


Figure. The TFC (%) from different morphological parts of yellow loosestrife

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**STUDY OF BIOMEDICAL PROPERTIES OF FRUIT
AND VEGETABLE POWDERS IN AN ANIMAL EXPERIMENT**

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Keywords: *jerusalem artichoke powder, rosehip fruit powder, clinical and physiological indicators, laboratory animals.*

Introduction. Among the factors of a healthy diet that are important for maintaining human health, efficiency and active longevity, the most important role belongs to the supply of the body with the necessary nutrients (vitamins, minerals, dietary fiber). Jerusalem artichoke has a positive effect on digestion, increases the resistance of the immune system. Rosehip fruits help reduce the risk of atherosclerosis, normalize metabolic processes. A promising way to preserve the beneficial properties of vegetable raw materials is drying at low temperatures with simultaneous grinding, which allows to obtain fine powders [1, 2].

Aim of the Study. The aim is to study the physiological effect and toxicity of jerusalem artichoke and rosehip powders in an experiment on laboratory animals.

Materials and methods. The study was conducted on clinically healthy white Wistar rats. The experiment involved groups of animals to which powders of 3% of the weight of the diet were introduced into the diet: group 1 – jerusalem artichoke powder; group 2 – rosehip powder and a control group (a diet without vegetable powders). Clinical and physiological parameters were evaluated daily, weighing – every 4th day. Activity testing in the “open field” and a physical activity tolerance test were carried out before the experiment, on the 21st and 28th days. The general clinical examination of blood samples was carried out with an automatic veterinary hematology analyzer. The content of minerals was determined by atomic absorption spectroscopy, vitamins – by high-performance liquid chromatography.

Results and discussion. When consuming jerusalem artichoke powder, the weight of animals increased (by 24.9%) compared to the control group, activation of the immune component of the blood was observed, a positive effect on the gastrointestinal tract, improvement of the pancreas. The value of the index of tolerance to physical activity increased by 20% compared to the control group. When animals consumed rosehip powder, the weight increased by 25.4% of the initial weight. The blood test showed increased hematopoietic function, increased secretory-motor function of the stomach, intestines, activation of protein metabolism. When eating the studied powders of toxic effects, as well as any negative deviations in the condition of the animals were not detected. There was a high content of vitamin A, C in rosehip powder, and in jerusalem artichoke powder – magnesium, calcium, iron.

Conclusions. Thus, the conducted studies allow us to recommend jerusalem artichoke and rosehip powders as a source of important macro- and micronutrients in the production of food additives and food products.

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**STUDY OF NEW PHARMACOLOGICAL PROPERTIES OF A PLANT EXTRACT
FROM THE HERB OF *PRIMULA VERIS L.***

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Keywords: *ГЭТТИБ, alcohol, heart, mitochondria.*

Chronic alcohol intoxication (CAI) induces heart damage. One of the promising ways of its treatment involves the administration of herbal medicinal products. The purpose of this study was to explore the effect of solid herbal extract of *Primula veris L.* (PVSHE) on the morphofunctional changes in rats' myocardium after exposure to chronic alcohol intoxication.

Introduction. Alcohol reduces the membrane potential of mitochondria, the activity of respiratory chain complexes, and also damages mitochondrial DNA. Therefore, the search and development of new methods for correcting myocardial damage caused by chronic alcohol intoxication are relevant.

Aim of study. The purpose of this study was to explore the effect of solid herbal extract of *Primula veris L.* (PVSHE) on the morphofunctional changes in rats' myocardium after exposure to chronic alcohol intoxication.

Materials and methods. CAI was simulated for 24 weeks. Loading testing was used to assess the functional condition of the heart, the functional assessment of mitochondria was based on the protocol described by I. R. Lanza and determination of the indices of lipid peroxidation and activity of antioxidant enzymes in cardiac mitochondria. We performed a microscopic examination of the left ventricle following the standard protocol of histological processing and hematoxylin and eosin staining.

Results and discussion. PVSHE restricts the toxic effects of ethanol on the heart which was indicated by a higher rise in the rates of myocardial contraction and relaxation, LVP and MISP when functional tests were conducted. PVSHE caused an improvement in the functional state of rats' cardiac mitochondria exposed to CAI, which was demonstrated by on average 1.3-1.4 times as high RCR as compared to the control group. This is consistent with the previously identified positive effect of PVSHE on the functional state of mitochondria, limiting the development of oxidative stress, increasing SOD activity [1, 2]. The histological examination of the myocardium of the animals treated with PVSHE showed the lack of tortuosity, an increase in the volume fraction of cardiac myocytes, and a 31.2% decline in the interstitial volume. Therefore, PVSHE has a protective effect on the heart after CAI exposure.

Conclusions. PVSHE has a protective effect on the heart after CAI exposure.

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**DEVELOPMENT AND STANDARDIZATION OF A PHYTOSUBSTANCE BASED
ON THE RAW MATERIALS OF *ORTHILIA SECUNDA* AND *RHODIOLA QUADRIFIDA***

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Keywords: *Orthilia secunda*, *Rhodiola quadrifida*, phytosubstance, standardization, validation.

Introduction. Comprehensive phytochemical study and standardization of the herb *Orthilia secunda* and rhizomes and roots of *Rhodiola quadrifida* created the prerequisites for the development of a phytocomposition based on them with a directed action for the treatment of gynecological pathologies [1,2]. As an optimal dosage form, a herbal collection was proposed, since the collections are the drugs of choice and occupy a significant share (31%) in the herbal medicine market [3]. In addition, the collection will make it possible to preserve the multicomponent composition of biologically active substances of the objects under study and develop standardization criteria using the quality parameters of the feedstock, which ensures end-to-end standardization, an approach adopted in modern pharmaceutical analytics.

Aim of study. To develop the optimal composition of the herbal preparation from the raw materials of *Orthilia secunda* and *Rhodiola quadrifida*, select the criteria for its standardization and conduct a validation and verification assessment of the analytical methods used.

Materials and methods. The objects of study were 3 variants of plant composition: «rhizomes and roots of *Rhodiola quadrifida* – herb of *Orthilia secunda*» in various proportions – 1:1, 2:1, 1:2. Water and alcohol extracts of the indicated phytocompositions were directly studied. Quantitative determination of salidroside and tyrosol was performed by HPLC (FlexarFX-15 chromatograph with UV detector (PerkinElmer, USA), Zorbax C18 column, 250 x 4.6 x 5 µm). Their identification and quantification was carried out using solutions of the corresponding standard samples produced by Sigma-Aldrich, TraceCERT®, Merck, LGC, PerkinElmer, and TsSOVV. The content of gallic acid (GA) was determined by the UV-SPM method on an SF-2000 instrument (ZAO OKB Spektr, St. Petersburg). Pharmacopoeial quality indicators were established according to the methods specified in the RF SP XIV [4].

Results and discussion. Based on the results of a phytochemical study of plant compositions in three variants of the ratios of the types of raw materials of *Rhodiola quadrifida* and *Orthilia secunda* (1:1, 1:2 and 2:1). Evaluation and subsequent selection of the optimal ratio was carried out according to the representativeness of the phytochemical composition. The optimal ratio of components was determined – 2:1. The content of marker components of individual plants in the composition of phytocomposition was established, which in the aqueous extract was: salidroside – 0.53%, tyrosol – 0.10%, GA – 4.22%.

The UV-SPM method was adapted to quantify GA in the herbal preparation. Its validation tests were carried out on the characteristics of specificity, precision, linearity, accuracy and robustness. All established validation parameters meet the acceptance criteria. Numerical indicators, technological parameters were determined for the phytocomposition, as well as characteristic macro- and microscopic features were established.

Conclusions. The optimal composition of the phytosubstance is proposed on the basis of raw materials of *Orthilia secunda* and *Rhodiola quadrifida*, and the criteria for its standardization were established. The pool of experimental data showed that the proposed methods give reproducible and reliable results and can be used to control the quality of the herbal collection. On the basis of the research carried out, a draft normative document «Collection gynecological № 1» was developed.

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PROSPECTS FOR THE USE OF THE DSC METHOD IN THE ANALYSIS OF THE QUALITY OF THE USE OF FISH OIL PREPARATIONS FOR MEDICINE AT A PRODUCTION SITE**Lopatin Vasily** (ORCID: 0000-0002-6543-0904), **Fetisova Angelica** (ORCID: 0000-0001-8077-0520)

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Abstract. *A study was conducted to analyze the quality of fish oil-based medicinal products for medical use using the DSC method. It was found that DSC produces clear thermogram graphs that do not differ from each other in the analyzed samples within the same production site, which can be used in production as an additional method for analyzing the quality of fish oil products for medical use.*

Keywords: *differential scanning calorimetry, thermal analysis, DSC, fish oil, quality analysis.*

Introduction. There are several directions of methods for assessing the quality of fish oil substances that are used in world practice, each of which has its own advantages and disadvantages [1-2]. One of them is thermal analysis, in particular the DSC method, which is used to determine thermal and oxidative stability, as well as to establish crystal polymorphism, melting point, cold crystallization, glass transition [3-4].

Aim of study. To study the temperature characteristics of domestic and foreign fish oil medicinal products for medical use by differential scanning calorimetry (DSC) to assess the quality at the manufacturing enterprise.

Materials and methods. Object – fish oil, purified for internal use of Russian production (series A and B) and fish oil-Teva (series C and D). Mettler Toledo DSC3+ Excellence Differential Scanning Calorimeter. A sample of fish oil, accurately weighed about 10 mg, was placed in aluminum crucibles with a volume of 40 mL. The analysis was carried out in a nitrogen atmosphere at a flow rate of 50 mL/min. Temperature program: [1] 25.0... -80.0 C°, -3.00 K/min, N2 50.0 ml/min; [2] -80.0 C°, 10.00 min, N2 50.0 mL/min; [3] -80.0...50.0 C°, 5.00 K/min, N2 50.0 mL/min.

Results and discussion. Analysis of domestically produced fish oil showed that, peaks corresponding to thermal effects were identified in the negative temperature range: from minus 60.14 C° to minus 12.02 C°. Comparing the DSC curves, it follows that the number of peaks on the thermograms of samples of series A coincides with the number of peaks on the thermograms of samples of series B. In the analysis of Teva fish oil, peaks corresponding to thermal effects, identified also in the negative temperature range: from minus 41.35 C° to minus 6.17 C°. Comparison of the DSC curves shows that the number of peaks on the thermograms of the C series samples coincides with the number of peaks on the thermograms of the D series samples. However, unlike the DSC curves of the domestically produced fish oil preparation, the DSC curves of the Teva fish oil drug have a more pronounced deviation from the baseline and a steeper slope of the peak, which indicates that the drug is more purified from impurities and related substances.

Conclusions. The analyzed dosage forms of domestic and foreign manufacturers give clear thermogram graphs and coincide within the same manufacturer. This makes it possible to use the DSC method as a quality control method for drugs based on fish oil substance at a specific production site.

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EFFECT OF ADDITION OF ACIDS ON AQUEOUS-ORGANIC EXTRACTION OF HYDROXYCINNAMIC ACIDS FROM DANDELION ROOTS**Lukashou Raman, Gurina Natalia**Belarusian State Medical University
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The article presents the results of evaluating the effect of adding acids during aqueous-organic extraction on the yield of hydroxycinnamic acids (HCA) from dandelion roots into the extractant.

Keywords: addition of acids, water-organic extraction, dandelion roots.

Introduction. Pre-treatment of medicinal plant materials, as a rule, is carried out before the extraction process. However, it can also be carried out during the extraction process. Such approaches include the addition of acids to the extractant.

Aim of study. To study the effect of the acids nature and concentration added to the extractant on the yield of hydroxycinnamic acids (HCA) from dandelion roots into the extractant.

Materials and methods. The object of the study was dandelion roots produced by LLC “NPK Biotest” (Republic of Belarus). For the extraction of HCA, a mixture of propanol-2, acetone, and water (50:10:40, by volume) selected earlier was used [1]. Inorganic (hydrochloric, sulfuric, phosphoric, nitric) and organic (formic, acetic) acids were added to the extractant. After selecting the acid, the range of its concentrations was studied from 2 % to 10 % with a step of 2 %.

Results and discussion. The figure shows the dependence of the HCA content on the nature of the acid added to the extractant.

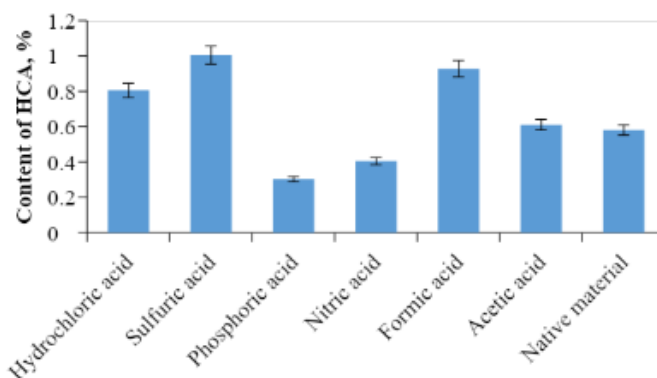


Fig. Dependence of the HCA content on the acid nature

When using hydrochloric, sulfuric, formic and acetic acids, the HCA content increased by 5.14–72.9 % (rel.). A statistically significant increase was noted for sulfuric, formic and hydrochloric acids. At the same time, no statistically significant difference was observed between sulfuric and formic acids. However, later sulfuric acid was used, because formic acid is volatile and irritating.

In the range of sulfuric acid concentrations from 2 % to 6 %, an increase in the HCA content from 2.08 to 2.37 times was observed with a maximum at 6 %. No statistically significant difference was observed between the concentrations of sulfuric acid, therefore, taking into account the economy of the reagent, it is recommended to use an addition of 2 %. At a concentration of 8 %, the content did not significantly differ from native material, and at 10% it sharply decreased by 4 times.

Conclusions. The addition of sulfuric acid in a volume concentration of 2% to a water-organic extractant leads to an increase in the release of HCA from dandelion roots.

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**PHYTOPHARMACEUTICALS FROM ORNAMENTAL RESOURCES:
FROM TRADITIONAL MEDICINE TO BIODISCOVERY PLATFORMS****Mahdi Ayyari^{1*}, Ghader Ghasemi¹, Alexander Crawford², Mohammad-Taghi Ebadi¹, Mohammad-Hossein Azimi³**¹ Department of Horticultural Sciences, Tarbiat Modares University, Tehran, Iran² Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway³ Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute, Agricultural Research, Education and Extension Organization, Mahallat, Iran

Introduction. Some medicinal plants are frequently used as an ornamental plants in urban spaces while interestingly, a few ornamental plants also got attention to produce bioactive metabolites. *Iris germanica* in addition to be an ornamental plant with a rainbow of flower colors, has been proposed as herbal drug in traditional medicine. Orris root is the rhizome of *I. germanica* has been evaluated for some neurodegenerative and skin disorder [1]. It has some bioactive compounds in essential oils and extract. The quality of its essential oil depends mainly on irone structures which are derived from triterpene iridals. According to the literature, irones are produced after 3 to 5 years of shelf life to be converted form the triterpenic iridals [2]. It is also a rich source of isoflavonoids.

Aim of the Study. A breeding plan for *Iris germanica* has been started in the Ornamental Plant Research Center (OPRC) in Mahallat, Iran, for producing different color type of *Iris germanica*, while the current research, led to profiling essential oils and isoflavonoids of 33 of *I. germanica* hybrids. Biodiversity will be evaluated in different hybrids among the examined samples. Some data also is offering the antiepileptic activity of orris root which could be verified using the biodiscovery platform of zebrafish epilepsy models and convert this myth or suggestion to a scientific reality or refuse it.

Materials and Methods. For the essential oil, dried powder rhizomes of *I. germanica* (50 g) were subjected to hydro distillation through a Clevenger apparatus for 3 h. One ml of ethyl acetate was added to collect the essential oil. The obtained essential oil was analyzed using GC-FID and GC-MS.

A methanolic extract was also obtained and each of the mentioned samples was powdered using a dry pulverizing mill (AG 500 g) and then the rhizome dried powder (1 g) was extracted by adding 20 ml of 99.9 % methanol using an ultrasonic device (120 Hz frequency) for 30 minutes at 30 °C and extracts were filtered using filter paper and used for identify isoflavonoids using HPLC and LC-MS.

Results and discussion. Based on the results of GC-MS analysis, 59 compounds were identified, 17 of which were major compounds. The highest amount of α -irone (25.52%) and β -irone (37.98%) compounds were observed in P5, one of the parent samples. Results also showed that the rhizome of *I. gemanica* is a rich source of isoflavonoids. Ornamental plants could be a promising source of some bioactive metabolites and the zebrafish biodiscovery platform can help to find more phytopharmaceuticals in them.

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THE USE OF IT IN THE TECHNOLOGY OF PHYTOSUBSTANCE
BASED ON THE ROOTS OF POLYGONATUM OFFICINALE

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Keywords: *the roots of Polygonatum officinale, polysaccharides, «Minitab» software.*

Introduction. Polygonatum officinale is a perennial herbaceous plant containing mucosal polysaccharides in its composition, which in the future make it possible to create a phytosubstantiation with an anti-inflammatory effect [1]. One of the methods for determining the content of polysaccharides is the spectrophotometric method with picric reagent [2]. Since there is no regulatory document for the roots of Polygonatum, there is a need for the selection of extraction parameters to determine the largest number of polysaccharides using the Minitab program [3].

Aim of this study. Consideration of the program for processing statistical data "Minitab" for the quantitative determination of polysaccharides in the roots of Polygonatum officinale.

Materials and Methods. Crushed roots of Polygonatum officinale were selected as the object of the study. The selection of optimal parameters (hydromodule and time) for quantitative determination is carried out using the spectrophotometric method with picric reagent using the program for static data processing "Minitab".

Results and discussion:

Thanks to statistical software, it is possible to create an experiment plan, identify the dependence of the polysaccharide content on the parameters under study, select optimal conditions and obtain the maximum value of polysaccharides. In addition, it is possible to provide statistical processing of the results and visualize the study. All this allows you to reduce the time for conducting research and minimize possible errors.

Conclusion. The program "Minitab" is proposed for the purpose of quantitative determination of polysaccharides in the roots of Polygonatum officinale.

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**APPLICATION OF EXOGENOUS AMINO ACIDS AND THE SECONDARY METABOLITES CONTENT
IN MOLDOVIAN SNAKEHEAD**

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Moldavian snakehead (*Dracocephalum moldavica* L.) is a promising aromatic crop containing a large list of pharmacologically significant compounds (Malankina, 2020). Increasing the content of phenolic compounds and essential oil in raw materials by using environmentally friendly preparations will improve the raw materials quality and the yield of target pharmacologically significant substances (Malankina, 2018).

The studies were carried out on the snakehead cv. Gorynych. Was used fertilizer OMEK® UNIVERSAL (Bioamid JSC, Russia) which has proven itself well on rapeseed, cereals, and sunflower. The active substances are organic chelate forms of trace elements in the form of L – aspariginates. It is recommended to increase the yield and its quality. Fertilizer Aminozol (Germany) contains a complex of amino acids and trace elements in a chelate form and has proven itself well on vegetable crops.

Plants were treated with a solution of the fertilizer in the phase of 8-10 leaves and in one other variant in 2 weeks after the first treatment. The crop was harvested in the phase of full flowering. The content of essential oil was determined in fresh raw materials according to the State Pharmacopeia of the Russian Federation. The content of flavonoids recalculated on rutin, the phenols and tannins content recalculated on gallic acid was determined spectrophotometrically.

The results are presented in table 1.

Table 1 – Effect of OMEK-Universal and Aminozol on the yield and biochemical parameters of the raw material of Moldavian snakehead

Variant	Yield, g/m ²	Essential oil, %	Flavonoids,%	Polyphenols, %	Tannins, %
Control	654	0,26±0,04	2,84±0,06	7,95±0,07	1,43±0,02
OMEK-Universal 0.5 g/l	616	0,33±0,05	2,82±0,05	8,13±0,11	1,18±0,04
OMEK-Universal 0.5 g/l x2	648	0,35±0,04	2,77±0,03	8,25±0,09	1,54±0,03
Aminozol 1 ml/l	614	0,28±0,03	2,62±0,05	8,41±0,04	2,81±0,04
Aminozol 1 ml/l x2	679	0,34±0,02	3.12±0,07	7,60±0,06	1,05±0,03

With a slight change in yield, the content of essential oil in raw materials increased by 19-34%. The treatment with fertilizer Aminozol 2 times with an interval 2 weeks increase the content of polyphenols by 10%. The content of flavonoids varied insignificantly.

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EFFECTS OF HERBAL MEDICINE FOR PREMENSTRUAL SYNDROME

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Keywords: *pms, herbs, the salicylic acid.*

Introduction. Premenstrual syndrome (PMS) is a recurrent luteal phase disorder characterized by irritability, agitation, emotional lability, depression, edema, breast pain, and headaches that occurs during the luteal phase of the menstrual cycle and usually ends a few hours after the onset of menstruation. Hyperprolactinemia, circulating estrogen and progesterone levels, abnormal responses to estrogen and progesterone, excess aldosterone or antidiuretic hormone, serotonin deficiency are basis of pathophysiology of PMS.

In the pelvis during PMS increases the level of cytokines (IL-1,2,6,8), highly sensitive C-reactive protein (hs-CRP), antibody titers to Hsp27 (anti-Hsp27). The activity of inflammatory processes affects the formation of the corpus luteum and the production of progesterone at the proper level. The severity of PM depends on the activity of the inflammatory reaction. The mechanism of action of herbs: blocking pro-inflammatory enzymes COX1, COX2.

Some herbs contain derivatives of the salicylic acid compound, the mechanism of action of which is similar to NSAIDs. They are contained in the meadowsweet, raspberry, willow, violet, aspen, agrimony. Therefore, the use of these herbs reduces pain and discomfort in the abdomen and other symptoms.

Aim of study. Investigate the effectiveness of taking an infusion of anti-inflammatory herbs containing salicylates.

Materials and methods. We conducted a study involving 26 women from 21 to 42 years old with the main symptoms of PMS – abdominal pain and tenderness of the mammary glands.

All women drank 500 ml of water infusion per day from the 14th to the 30th day of the menstrual cycle, water infusion of meadowsweet, agrimony, raspberries, violets in equal proportions within one menstrual cycle.

Results. 89% of women noted a decrease in abdominal discomfort compared to the previous cycle without the use of infusion, 78% noted less tenderness and soreness of the mammary glands.

Conclusions. Herbs containing salicylates may be effective in reducing PMS symptoms.

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OPTIMIZATION OF THE TECHNOLOGY FOR OBTAINING ESSENTIAL OIL OF ORIGANUM VULGARE HERB

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Introduction. An important task in the development of technology for obtaining essential oil is to establish the optimal values of the parameters that have a direct impact on the yield of the final product. To solve it we used the design procedure response surface methodology according to the principle [1].

Purpose of research. The purpose of this study was to establish a set of optimal parameters for the extraction of essential oil from *origanum vulgare* herb.

Materials and methods. *Plant materials:* *Origanum vulgare* herb, collected in the district Esenyurt, Istanbul province, Turkey, 97 m above sea level.

Obtaining of essential oil was carried out according to the technology [2] at the ratio of plant materials and distilled water and distillation time according to Table 1.

Analysis of the essential oil: The analysis of the essential oil was performed according to a procedure similar to [2].

Results and discussion. To determine the effect of parameters on the yield of essential oil and, in particular, carvacrol, a matrix of experiments presented in Table 1 was compiled. The extractant to plant material ratio (X_1) and extraction time (X_2) were chosen as independent variables. The mass yield of carvacrol as the main active ingredient of the essential oil was used as the response. The surface plot is shown in Figure 1.

Table 1 – Matrix of experiments

Experiment (Run)	Independent variables		Response
	X_1 , mL/g	X_2 , min	Carvacrol yield, % w/w.
1	15	60	1,32
2	13	60	1,37
3	11	180	1,22
4	13	120	1,54
5	15	120	1,42
6	15	180	1,16
7	11	120	1,27
8	11	60	1,16
9	13	180	1,39
10	13	120	1,61
11	13	120	1,57
12	13	120	1,56
13	13	120	1,52

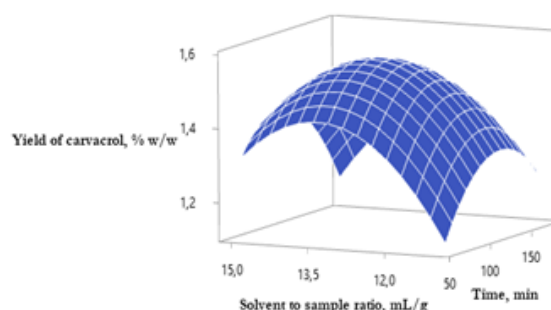


Figure 1. Response surface

Conclusion. The response surface of the yield of carvacrol from the variables presented above was established. It allows to determine the optimal parameters for carvacrol as the main active ingredient of the essential oil of *origanum vulgare* herb obtaining.

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CHALLENGES AND PROSPECTS FOR MEDICINAL PLANT PRODUCTION IN RUSSIA

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Introduction. According to estimates, the global trade value of plants used for medicinal purposes may surpass US\$ 165,66 billion and is progressively fueled by the demand from various industries. Plants used in medicine are important not only in local health practices, but also in global trade due to their extensive commercial use and value. [1,2].

Aim of study. The aim of the study is to investigate and evaluate the current state of medicinal plant production in Russia, with a focus on identifying the challenges and opportunities that exist in this field.

Materials and methods. The study used methods such as content analysis, aggregated data, and comparative analysis. The information sources for the research included the DSM Group database, AlphaRM database, and data from the Federal State Statistics Service and the Federal Customs Service of the Russian Federation.

Results and discussion. At present, the market volume of phytomedicine in the overall pharmaceutical market of Russia amounts to 833.8 million dollars, which is less than 4% of the total market. The acreage of cultivated land dedicated to medicinal crops and essential-oil crops amount to 9.9 thousand hectares and 90 thousand hectares, respectively. Simultaneously, there is a trend towards a 35% reduction in the area under medicinal crops in 2022 compared to 2021. Furthermore, in Russia, there exist approximately 194 commercial organizations that are involved in the gathering and harvesting of non-timber forest resources, food forest resources, and medicinal plants. The average annual imports of medicinal plant material into Russia amount to \$32 million, equivalent to 10477 tons. It is noteworthy that the imports comprise species that are conventionally cultivated and harvested in Russia, such as chamomile and marigold flowers imported from Egypt, rosehip fruits from Chile, and valerian rhizomes and roots and dandelion roots from China. In contrast, the mean yearly exports of medicinal plant material from Russia sum up to 3633 tons, valued at 13 million dollars. Notably, licorice roots make up the primary export, accounting for about 3% of the total exports.

Currently, there exist several significant barriers to the growth of the Russian market of medicinal herbal raw materials. These obstacles include the absence of domestic seed stock of medicinal plants, loss of expertise and knowledge in herb cultivation, inadequate modern equipment for large-scale cultivation, harvesting, and processing of herbs, insufficient numbers of agrotechnics and primary processing specialists for medicinal raw materials, and a dearth of clinical studies on herbal medicines. A potential solution to this issue may involve the establishment of a state entity responsible for coordinating and overseeing the cultivation of medicinal crops, as well as providing efficient state aid to agricultural producers.

Conclusions. In summary, the market for medicinal herbs in Russia exhibits export potential. However, several challenges are hampering the growth of the industry, including a lack of indigenous seeds, declining knowledge of herbal cultivation, inadequate equipment and expertise for large-scale cultivation and processing, and dwindling areas devoted to medicinal plants. To maximize the export potential of medicinal herbs, it is imperative to address these challenges. Overcoming these hurdles could position Russian producers at the forefront of the rapidly expanding global market for medicinal herbs.

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ALKALOID DETERMINATION IN DRUG «VERATRUM AQUA» BY HPLC-MS/MS

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E-mail: melnik_e_v_2@staff.sechenov.ru**Keywords:** *veratrum aqua, veratrum, alkaloids, HPLC-MS/MS.*

Introduction. State of the art is relevant study of the alkaloid composition of «Veratrum Aqua» for the purposes of standardization of this drugs of different manufacturers.

Aim of study. To develop a methodology for the determination of the main alkaloids by HPLC-MS/MS.

Materials and methods. Three main alkaloids: jervine, protoveratrine A and protoveratrine B have been identified in veratrum aqua by developed method high performance liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS). Previously, all samples of «Veratrum Aqua» have been filtered through a membrane filter followed by 10-fold dilution with a mixture of acetonitrile/ water (1:2).

Chromatographic separation was carried out on columns ke Poroshell 120 EC-C18 (4.6 × 100 mm, 2.7 μm) with Poroshell 120 EC-C18 column (4.6 x 5 mm, 4 μm, both Agilent Technologies, USA) with a temperature 40°C. The injection volume was 2 μL. Time of analysis – 14 min. Solvent A of the mobile phase – 0.1% formic acid and 5 mM ammonium formate in ultrapure water; solvent B – 0.1% formic acid in acetonitrile. The gradient elution was performed at a constant flow rate of 0.8 mL/min. Solvent B changed from 10% – 0 to 1 min; rapidly increased to 25% until 1.1 min and maintained at 25% B to 1.5 min; linearly increased to 50% at 9.5 min then to reach 90% at 9.6 min until 11 min, rapidly returned to 10% B at 11.1 min and then maintained until 14 min. The retention time of jervine was 6.93 min, proA—9.24 min, proB—7.58 min. Product ions and collision energy of Veratrum alkaloids were include to the table.

Table – Product ions and collision energy of Veratrum alkaloids

Alkaloid	Precursor ion	Product ions (collision energy)
Jervine	426.2	114.1 (36)/109.1 (36)/84.1 (44)
Protoveratrine A	794.2	776.1 (44)/758.1 (44)/658.1 (56)
Protoveratrine B	810.4	792.5 (40)/676.5 (70)/658.5 (60)

Conclusion. The methodology of determination of alkaloids: jervine, protoveratrine A and protoveratrine B was developed by HPLC-MS/MS in drug «Veratrum Aqua» for further researches and standardization of this drugs.

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**FABRICATION OF FUCOIDAN/CHITOSAN NANOPARTICLES FOR ENHANCED DELIVERY OF PIPERINE.
A STUDY OF ITS ANTIOXIDANT ACTIVITY****Mensah E.O.¹, Mironov, M.A.¹**¹Institute of Chemical Engineering, Ural Federal University
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Introduction. Piperine is an alkaloid present in pepper, mostly prominent in black pepper. They are widely known for an array of biological activities such as anticancer, anti-inflammatory, antioxidant, cardioprotective, and antiaging activities [1]. As such they have been used in the food, pharmaceutical, and cosmetic industries for development of beneficial products, however, the bioavailability of piperine is its major drawback.

Aim of the Study. As such, the main aim of the study was to develop a nanoparticle for the encapsulation of piperine to improve its bioavailability *in vitro*.

Materials and methods. Nanoparticles from fucoidan and chitosan were prepared by the polyelectrolyte self-assembly method [2]. Nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), and atomic force microscopy (AFM) for selecting an ideal for encapsulation of piperine. An *in vitro* study of piperine was carried out using the dialysis method in both acidic and neutral media [1]. Antioxidant activity of the piperine-loaded nanoparticles and piperine (at concentrations, 12.5, 25.0, 50.0, 100.0, and 200.0 µg/mL) were tested against DPPH and hydroxy radical scavenging assay with ascorbic acid as the positive control.

Results and discussion. The nanoparticle carrier from fucoidan and chitosan were successfully fabricated with size of 219.67 ± 0.61 nm and a zeta potential of -17.30 ± 1.07 mV. Results from the FTIR further confirmed a formulation of the nanoparticle where functional groups of both fucoidan and chitosan were prominent. After loading of piperine, the size significantly increased ($p < 0.05$) to 355.63 ± 14.49 nm and having a surface charge of -21.17 ± 0.68 mV. This indicates a low tendency of aggregation of nanoparticles. Also, the amount of piperine loaded into the fucoidan/chitosan nanoparticle was determined and found to be 92.78 ± 0.02 %. Additionally, the release profile of piperine revealed a faster release of piperine from the nanoparticles as compared to the unformulated piperine. Furthermore, the piperine-loaded nanoparticles exhibited good antioxidant activity against DPPH and HRSA assay at all concentrations.

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**APPLICATION OF WATERCRESS (*LEPIDIDIUM SATIVUM* L.) IN DETERMINING
OF THE ANTIBACTERIAL MEDICINES ECOTOXICITY**

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Introduction. One of the standard test objects for a comparative assessment of the medicines toxicity can be a dicotyledonous watercress (*Lepidium sativum* L.). Watercress is a bioindicator, characterized by rapid seed germination, good germination, which is reduced in the presence of toxic compounds [1].

Aim of the Study. to study the ecotoxic effect of antibacterial drugs using watercress (*Lepidium sativum* L.).

Materials and Methods. We used the seeds of watercress (*Lepidium sativum* L.) We studied the effect on seed germination of ciprofloxacin substance 1%, ampicillin sodium substance 1%, amoxicillin substance with clavulanic acid 1%, gentamicin sulfate substance 1% solutions, water-alcohol suspension of azithromycin substance 1% (volume ratio of purified water and ethyl alcohol 96% 8:2). As a control, purified water and a solution of purified water and ethyl alcohol 96% in a ratio of 8:2 were used. 30 watercress seeds were placed in Petri dishes, sterile cotton wool was used as a substrate for seed germination. Seeds were watered with a standard volume of test solutions (2.5 ml) for 6 days.

Results and Discussion. The results of the experiment are presented in table.

Table – The number of germinated seeds (hereinafter referred to as GS) and the number of seeds that have developed into a green plant (hereinafter referred to as GP)

	Day 1 – day 2	Day 3	Day 4	Day 5	Day 6
Purified water (control)	0	30 GS	3 GS/27 GP	28 GP	28 GP
Solution of purified water and ethyl alcohol 96% in a ratio of 8:2 (control)	0	0	0	0	0
Solutions of ciprofloxacin substance 1%	0	27 GS	26 GS/3 GP	0	0
Solutions of ampicillin sodium substance 1%	0	27 GS	2 GS/25 GP	25 GP	25 GP
Solutions of amoxicillin substance with clavulanic acid 1%	0	26 GS	11 GS/17 GP	14 GP	14 GP
Solutions of gentamicin sulfate substance 1%	0	23 GS	23 GS	0	0
Water-alcohol suspension of azithromycin substance 1%	0	0	0	0	0

It was found that the percentage of seed germination of watercress was lower when a solution of antibacterial drugs was added to the substrate in comparison with the control. When adding a water-alcohol suspension of azithromycin 1% and a water-alcohol solution (control) to the substrate, watercress seeds did not germinate. With the addition of a solution of ampicillin sodium 1% and a solution of amoxicillin with clavulanic acid 1%, watercress seeds germinated and developed to green plants, however, they lagged behind the control (purified water) in the rate of development. Solutions of ciprofloxacin 1% and gentamicin sulfate 1% showed the highest ecotoxicity; after germination, by the 5th day of observation, all plants died.

Conclusions. The studied medicines had ecotoxicity in the experiment. Solutions of ampicillin sodium and amoxicillin with clavulanic acid slowed down the development of green watercress plants. When solutions of ciprofloxacin 1% and gentamicin sulfate 1% were added to the substrate, green plants did not develop from seeds.

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**INVESTIGATING IN-VITRO ANTICANCER PROPERTIES
OF ETHNOMEDICINAL PLANTS OF BANGLADESH**

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Keywords: *Ethnomedicinal plants, indigenous communities, cytotoxicity, antitumor activity, apoptosis pathway, Stat3 and Akt pathway, Bangladesh.*

Abstract. The traditional healers of indigenous communities of Bangladesh use several plants/plant parts, with often unknown ingredients, for the treatment of cancer/tumors, often with promising anecdotal results. To document such traditional usage, we interviewed more than 1000 informants of 12 indigenous communities of Bangladesh and documented more than 5000 using information of medicinal plants from them. To date, we have recorded 1479 Bangladeshi medicinal plants for the first time, of which, only a small portion were screened for their anticancer properties. To investigate such traditional treatments, we selected ten ethnomedicinal plants for *in-vitro* studies. We performed the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on a panel of cancer cell lines to evaluate cytotoxicity of the hydroethanolic leaf/stem/flower extracts at six different concentrations. Preliminary screening results showed that the extent of inhibition varied between the types of cell lines and also the types of extract used. Out of 15 plant parts of the ten species tested, some of them were active and showed > 50 % cytotoxic activity against the different cancerous cell lines. A total of 40 compounds were identified from the active extracts through GC-MS technique and three compounds were isolated and identified through HPLC and NMR. Among the isolated compounds, compound-2 significantly decreased the ratio of Bcl-2/Bax and increased the expression level of cleaved caspase-9 and -3 in a concentration-dependent manner. Together, these results proved that compound-2 had the potential to induce apoptosis in U-251 cells by activation of the intrinsic/mitochondrial pathway. In addition, compound-2 also suppressed the activity of Stat3 and Akt, postulating that compound-2 induced apoptosis might be triggered by the inhibition of Stat3 and Akt expression. The results of this study support the possible use of this ethnomedicinal plant in the treatment of tumors/cancers, and support its traditional use. To the best of our knowledge, the antitumor/anticancer activity of the chosen species is reported herein for the first time.

THE SAMPLE PREPARATION AND ANALYSIS OF *DROSERA* TISSUE CULTURESMorshneva Alexandra^{1,2}, Grigorchuk Valeria¹, Khandy Maria^{1,2}, Gorpenchenko Tatiana^{1,2}¹Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS

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Introduction. The plant genus *Drosera* is one of the largest genera of carnivorous plants. *Drosera* extracts have antibacterial, antifungal and antitumor properties due to the content of flavonoids and naphthoquinones [1]. The natural habitat of this genus is shrinking, so the search and development of alternative technologies for obtaining biomass of *Drosera* with a high content of biologically active substances becomes relevant. Sample preparation methods are a complex process that determines the qualitative and quantitative composition of the final extract.

Aim of the Study. Evaluation of the influence of various methods of sample preparation on the biochemical composition of valuable groups of metabolites of cell cultures of *Drosera*.

Materials and Methods. Biochemical analysis of extracts of five lines of cell cultures was carried out: three long-term lines of *D. rotundifolia* – "Green light", "Green dark", "Red" and two primary lines of *D. capensis* obtained by us – "Light", "Dark". To obtain extracts, different methods of preparation of the raw material were used: grinding of fresh tissues with the use of nitrogen, drying with warm air (+40 °C), and lyophilization. Samples were extracted with 80% methanol. Determination of secondary metabolites was carried out by high performance liquid chromatography with ultraviolet and mass spectrometric detection.

Results and Discussion. As a result of biochemical analysis, twelve compounds were identified – naphthoquinones (rossoliside and plumbagin), flavonoids and their glycosides (myricetin-3-O- β -glucopyranoside I, myricetin-3-O- β -glucopyranoside II, hyperoside, isoquercitrin, myricetin and quercetin), ellagic acid derivatives (3-O-methylellagic acid glucopyranoside, 3,3'-di-O-methylellagic acid 4-O- β -D-glucopyranoside, 3,3'-Di-O-methylellagic acid) and epigallocatechin gallate. The results of the analysis of biomass extracts from fresh raw material without drying were not significant compared to dried biomass. The yield of substances is at least 2-3 times less. Most likely, this is due, firstly, to the water content of the cultures (70-80%), which increases the concentration of water during extraction with alcohol; secondly, losses during the concentration of extracts. Drying with warm air showed results comparable to lyophilization. However, the content of flavonoid glycosides, ellagic acid derivatives and naphthoquinones decreases. And at the same time, the content of flavonoid aglycones increases. The decrease in the content of glycosides in them can be mediated by the action of glycosidases. In addition, the grinding of the material dried by warm air in a mortar was more difficult than dried by lyophilization. Thus, the best results were obtained during the preparation of biomass by the method of lyophilization. The one showed a high yield of secondary metabolites. When comparing the results, the data obtained on the primary cultures of *D. capensis* were excluded, since a large spread of indicators between the repetitions of the samples was observed. Instability in the composition of secondary metabolites for primary lines is known from literature data [2].

Conclusions. According to the results of the study, it is recommended to use lyophilization to prepare the biomass of *Drosera* cell cultures for analysis.

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**PHARMACOLOGICAL EVALUATION OF CALOTROPIS PROCERA EXTRACTS:
A POTENTIAL SOLUTION FOR INFLAMMATION AND DIABETES**

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Abstract:

Aim. The negative side effects of synthetic medicinal drugs on patients have led to a demand for new drugs that can provide optimal treatment while minimizing these negative effects.

Material and methods. This research focuses on the medicinal properties of *Calotropis procera*, a commonly used plant in herbal medicine, to discover new anti-inflammatory, anti-diabetic, and anti-microbial drugs. In vivo experiments on rabbits and mice were conducted to evaluate the plant's efficacy.

Results. The aqueous extract of *C. procera* showed significant anti-inflammatory and anti-diabetic effects, with a dosage of 1000mg/kg body weight producing 52% inhibition in inflammation and a 500mg/kg body weight extract producing a $53.4\% \pm 4.62$ decrease in blood glucose levels in diabetic mice. The plant did not exhibit significant antimicrobial properties against various bacteria and fungi, except for *Staphylococcus aureus*, which was inhibited by all three organic extracts.

Conclusion. Overall, the results indicate that the aqueous flower extract of *C. procera* has potential as an effective treatment for inflammation and high blood glucose levels in animals.

ИЗУЧЕНИЕ КАРДИОТРОПНОЙ АКТИВНОСТИ ЭКСТРАКТОВ *ZIZIPHORA BUNGEANA* JUZ.

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Ziziphora bungeana Juz. – полукустарник из семейства Lamiaceae с характерным ароматом, произрастающий в Китае, Казахстане, Кыргызстане, Монголии, России, Таджикистане, Туркменистане и Узбекистане. Род *Ziziphora* L. (Lamiaceae) включает около 30 видов, широко распространенных в Азии, Африке и Европе.

Растения зизифоры используются в народной медицине как ранозаживляющее, антисептическое, седативное средство также, для лечения заболеваний сердечно-сосудистой системы, такие как ишемическая болезнь сердца и другие.

Экстракты, полученные из *Ziziphora bungeana* Juz. травы были подвергнуты фармакологическому скринингу для оценки их эффективности при гемической гипоксии. В результате скрининга на модели гемической гипоксии из 17 экстрактов были определены наиболее перспективные кандидаты – водный и ультразвуковой (60%) экстракты Зизифоры Бунге. После включения в основное исследование кандидатов, на крысах-самцах была смоделирована хроническая сердечная недостаточность и оценивалась кардиотропное действие выбранных экстрактов. По результатам проведенного исследования можно сделать вывод о выраженном кардиопротекторном эффекте испытуемых извлечений. Данные выводы подтверждаются результатами ЭхоКГ (достоверное увеличение ФВ по сравнению с группой патология без лечения), ЭКГ и гистологического анализа. Чтобы предположить, какие соединения могут быть потенциально ответственны за наблюдаемую фармакологическую активность, было проведено дальнейшее фитохимическое исследование экстрактов *Z. bungeana*.

В результате получены шесть ранее не описанных производных монотерпеноидов – Зизифорин А (1), Зизифорин В (2), зизифорозид D (3), 6'-малонилзизифорозид D (4), Зизифорозид E (5) и 6'-малонилзизифорозид A (6) наряду с одним ранее описанным монотерпеноидом – 7-гидроксипиперитон (7) и шесть полифенолов – пиноцембрин-7-О-рутинозид (8), хризин-7-О-рутинозид (9), акацетин-7-О-рутинозид (10), лютеолин-7-О-рутинозид (11), рутин (12) и розмариновая кислота (13) были выделены из *Z. bungeana* и их структура были описаны с помощью HR-ESI-MS, обширных экспериментов с 1D- и 2D-ЯМР, а также сравнения их спектроскопических данных с данными, представленными в литературе.

OBTAINING AND INVESTIGATION OF ARALIA CORDATA THUNB. CALLI

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Introduction. *Aralia cordata* is a perennial herbage plant, which has been listed in the Red book of the Russian Federation. Pharmaceuticals which are based on herbal substances of *Aralia cordata* have a valuable type of pharmacological activity and are widely used in oriental medicine [1]. Limitation of the natural geographic range and the combination of biological activities useful for humans make *Aralia cordata* Thunb. prospective object for in vitro introduction.

The aim of the study is obtaining of the viable cell culture of *Aralia cordata* Thunb. and investigation of its chemical constituents.

Materials and Methods. The primary explants for obtaining of *Aralia cordata* calli were pieces of leaves of the intact plant. Explants were sterilized with 2% benzalkonium chloride solution for 5 minutes. Induction of primary callusogenesis was provided on Murasige-Skoog medium with addition of 0,5 mg/l 2,4-D and the equal quantity of kinetin. Nutrient media with different constituents were discovered for choosing one for long-time cultivation of calli. Ethanol extracts from the intact plant and calli cultures (1:10) were assayed with HPTLC PRO SYSTEM (CAMAG AG, Switzerland).

Results and Discussion. The results of our experiments showed that optimal growth characteristics calli culture showed on Linsmaier-Skoog medium with addition of 1 mg/l 2,4-D and 0,25 mg/l of kinetin. Analysis of extracts from callus cultures by HPTLC demonstrated at least 5 compounds ($R_f = 0.15; 0.22; 0.38; 0.43; 0.64$), which were characteristic for all initial objects, two substances ($R_f = 0, 57; 0.89$), were characteristic for *Aralia* leaves and calli cultures. All found compounds belong to the class of triterpene glycosides. In addition, it was set, that chemical composition of calli remains constant over the time.

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**VALIDATION OF A HPLC METHOD FOR THE DETERMINATION OF PROTOCATECHUIC ACID
IN EXTRACTS OF *HIBISCUS SABDARIFFA***

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Keywords: *Hibiscus sabdariffa*, protocatechuic acid (APC), polyphenol, validation, HPLC/

Introduction. *Hibiscus sabdariffa* L., belonging to the family of *Malvaceae* – contains the main substances are flavonoids, polyphenols with many typical effects such as: antioxidant, anti-inflammatory, anti-cancer, protective liver, lower blood pressure... [1].

There have been various studies on the quantification of polyphenol content from *Hibiscus sabdariffa*. To do that, the study made a new point which is the quantification of protocatechuic acid (APC) from *Hibiscus sabdariffa*.

Aim of the Study. In this study, we have built a quantitative procedure for APC in *Hibiscus* extract by HPLC method and verified that the quantitative procedure is reasonable

Materials and Methods. By HPLC method, fixed conditions: Chromatographic column (Phenomenex Gemini C18 – 250 mm x 4.6 mm; 5 µm); Detector: PDA; Column temperature (30°C); Injection volume (10 µl); Quantitative wavelength (260 nm). Investigate mobile phase solvent system, choose 2 systems: (1) A – Formic acid 0.1% and B – ACN; (2) A – trifluoroacetic acid 0.1% and B – ACN; Gradient if A: 85%-60% only B: 15%-40% for 35 minutes. Investigate flow rate: 1ml/min and 0.6ml/min after select the mobile phase.

Quantified APC by the standard curve method. Dilute APC standard 50 µg/ml with MeOH to obtain a range of concentrations: 1,0-2.5-5.0-10.0-20.0 µg/ml.

Validation of the APC quantification method in the extract of *Hibiscus* according to the guidelines of ICH [2] on System Suitability Testing, Specificity, Linearity, Repeatability, Accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Results and Discussion. Built a process to evaluate APC in *Hibiscus* extract by HPLC method with chromatographic conditions including: column Phenomenex Gemini C18 (250 mm x 4.6 mm; 5 µm), PDA detector, Column temperature (30°C); Injection volume (10 µl); Quantitative wavelength (260 nm), flow rate 0, 6 ml/min, mobile phase (2): A – trifluoroacetic acid 0.1% and B – ACN; Gradient if A: 85%-60% only B: 15%-40% for 35 minutes.

The method has been validated, the analytical system is suitable, the process is specific, there is a close linear correlation between peak area and APC concentration in the range (1,02-20,4 µg/ml), $R^2 = 0,9984$; high accuracy (average 99, 80%) and high repeatability (RSD < 2%); The LOD and LOQ were 0.03 µg/ml and 0.10 µg/ml, respectively.

This method has been applied to further studies on the preparation of dried extracts of *Hibiscus sabdariffa*.

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DETERMINATION OF ALISOL-A IN *ALISMA RHIZOMA* EXTRACTS BY HPLC METHOD

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Introduction. *Alisma rhizoma* is a precious medicine widely used in traditional medicine of Vietnam. Triterpenoids are considered to be the major biologically active ingredients in *Alisma rhizoma* [1], including Alisol-A. Therefore, this study developed a procedure for the quantification of Alisol-A (AA) in *Alisma rhizoma*.

Aim of the Study. In this study, we have built a quantitative procedure for AA in *Alisma rhizoma* extracts by HPLC method and verified that the quantitative procedure is reasonable

Materials and Methods. By HPLC method, fixed conditions: Chromatographic column (Agilent C18 – 250 mm x 4.6 mm; 5 μ m), detector: PDA, column temperature (30°C), injection volume (10 μ l), flow rate: 0,75 ml/min, quantitative wavelength (210 nm). Investigate mobile phase solvent system, choose 3 systems: (1) ACN : HCOOH 0,1% (75:25); (2) ACN : H₂O (75:25); (3) ACN : H₃PO₄ 0,1% (75:25)

Quantified AA by the standard curve method. Dilute AA standard 490 μ g/ml with MeOH to obtain a range of concentrations: 49, 98, 147, 196, 245 μ g/ml.

Validation of the AA quantification method in the extract of *Alisma rhizoma* according to the guidelines of ICH [2] on System Suitability Testing, Specificity, Linearity, Repeatability, Accuracy, Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Results and Discussion. Built a process to evaluate AA in *Alisma rhizoma* extract by HPLC method with chromatographic conditions including: column Agilent C18 (250 mm x 4.6 mm; 5 μ m), PDA detector, column temperature (30°C), injection volume (10 μ l); Quantitative wavelength (210 nm), flow rate 0,75 ml/min, mobile phase (1) ACN : HCOOH 0,1% (75:25).

The method has been validated, the analytical system is suitable, the process is specific, there is a close linear correlation between peak area and AA concentration $R^2 = 0,9986$; high accuracy (average 100.91%) and high repeatability (RSD < 2%); The LOD and LOQ were 0,26 μ g/ml and 0,86 μ g/ml, respectively.

This method has been applied to further studies on the preparation of dried extracts of *Alisma rhizoma*.

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**PLATELET ACTIVATION AS BIOLOGICAL ACTIVITY MARKER
OF PLANT ORIGIN DIHYDROPHENANTHRENE DERIVATIVES**Orekhova I.A.¹¹St. Petersburg State Chemical and Pharmaceutical University
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Introduction. The biological and pharmacological effects of natural plants compounds are currently being actively studied. One of these compounds are dihydrophenanthrene derivatives, for which cytotoxic, antiproliferative effect and therefore anticancer, antimicrobial, antifungal activity has been revealed [1]. A number of dihydrophenanthrene derivatives were found in the cells of the medicinal plant *Empetrum nigrum*. This plant is known in folk medicine for its action on cardiovascular, metabolic pathologies, vitamin C deficiency and the study of the action of its metabolites will help determine their pharmacological value[3].

Platelets are nuclear-free blood cells that perform the important function of protecting the body from blood loss when the walls of blood vessels are damaged. The presence of numerous receptors in the platelet membrane ensures high reactivity of these signal acceptors when they exposed to physiological and non-physiological compounds [4].

Aim of the Study. The objective of this study was to analyse the use of blood platelet in vitro to determine the biological activity of dihydrophenanthrene derivatives of plant *Empetrum nigrum*.

Materials and Methods. Biological activity of dihydrophenanthrene derivatives isolated from the plant *Empetrum nigrum* is being cultivated in the lembolovsky nursery of medicinal plants at St. Petersburg State University of Chemical and Pharmaceutical Sciences was investigated. Studies were carried out by flow cytometry method using platelet rich plasma (PRP).

Results and discussion. Platelets are highly reactive cells able to initiate an extremely fast and sensitive response and model system with their application for evaluation different compounds activity is used in laboratory practice. At the first stage, the dose-dependent effect of 2,3,4-trimethoxy-5-hydroxy – 9,10-dihydrophenanthrene on the state of platelet was studied. Platelet were incubated with 1 – 30 mkM dihydrophenanthrene derivative for 20 min and tested. The fraction of platelet was tested against agonist ADP-stimulated human platelet activation and aggregation and significant and dose-dependent inhibitory action were found compared with free (control) platelet. Fraction-treated human platelet were tested under a Bechman coulter CytoFLEX, which shown the clear dose-dependent inhibition of platelet aggregation with increased 2,3,4-trimethoxy-5-hydroxy – 9,10-dihydrophenanthrene concentration, whereas the platelet treated with only the carrier and agonist (ADP) caused full platelet aggregation.

Conclusions. Our study was demonstrated that dihydrophenanthrene derivatives have biological activity detected on a model system using platelets as highly reactive cells. This investigation demonstrated that dihydrophenanthrene derivatives causes platelet inhibiting mediated by complex processes including procoagulant platelet formation.

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QUALITATIVE DETECTION OF FLAVONOIDS IN SHOOTS OF *CRATAEGUS PENNSYLVANICA* ASHE

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Keywords: *Crataegus pennsylvanica* Ashe, shoots, thin-layer chromatography, qualitative composition, flavonoids.

Introduction. Hawthorn is a plant of the Rosaceae family. It is used for diseases of the cardiovascular system, with weakening of the heart muscle after infectious diseases. It has the ability to dilate the vessels of the brain, helps with insomnia, dizziness [1]. The raw materials of hawthorn contain a high concentration of bioflavonoids, which have antioxidant, antihypoxic, hepatoprotective and many other pharmacological properties [2].

Aim of study. The aim of our study is to determine the qualitative composition of *Crataegus pennsylvanica* Ashe) shoots.

Materials and methods. The research material is shoots of *C. pennsylvanica*, collected in the spring of 2022, in the botanical garden of Ufa. We also used standard samples of flavonoids: rutin, quercetin, hyperoside, luteolin, apigenin, genistein, kaempferol, vitexin, naringenin, phisetin, luteolin-7-O-glycoside. Qualitative analysis of flavonoids was carried out by thin-layer chromatography on «Sorbfil UV254» plates (100 by 150, grain size 5-17 microns, «Imid», Krasnodar). After chromatography, the plates were treated with an alcoholic solution of aluminum chloride. The appearance of fluorescence under UV light was observed on the UVS–254/365 irradiator («Imid», Krasnodar) at a wavelength of 365 nm. Standard samples were used to identify flavonoids. We compared their mobility coefficients (Rf) with each other.

Results and discussion. The figure shows a schematic drawing of the resulting chromatogram. We compared the mobility coefficients and fluorescence, according to which flavonoids were found in the shoots: quercetin, rutin, hyperoside, luteolin, vitexin, luteolin-7-O-glycoside.

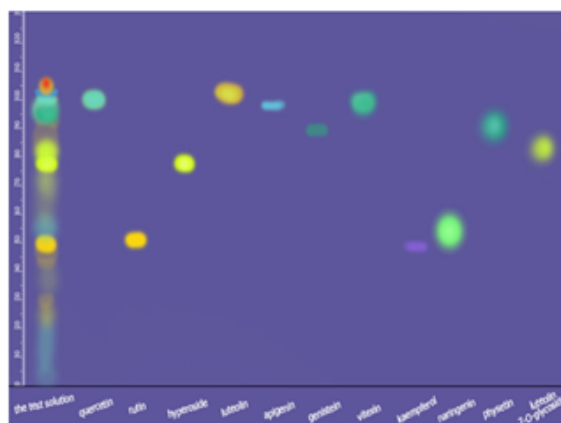


Figure. Schematic drawing of the resulting chromatogram

№	Flavonoid	R _f standard sample	R _f in the test solution
1	quercetin	0,90	0,89
2	rutin	0,46	0,45
3	hyperoside	0,71	0,71
4	luteolin	0,93	0,92
5	apigenin	0,89	-
6	genistein	0,81	-
7	vitexin	0,90	0,88
8	kaempferol	0,45	-
9	naringenin	0,49	-
10	phisetin	0,83	-
11	luteolin-7-O-glycoside	0,75	0,76

Conclusions. A qualitative analysis of flavonoids in the shoots of *C. pennsylvanica* was carried out. Flavonoids detected: quercetin, rutin, hyperoside, luteolin, vitexin, luteolin-7-O-glycoside.

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**USE OF PHYTOCOMPONENTS IN THE COMPOSITION
OF A COMBINED PREPARATION FOR HEPATOPROTECTIVE THERAPY****Perez G.B.¹, Abdrakhmanov A.E.¹, Protsyuk A.P.¹, Kaukhova I.E.¹**¹St. Petersburg State Chemical and Pharmaceutical University
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For individual or combined hepatoprotective therapy, extracts of plants such as milk thistle, artichoke, mint and meadowsweet are used. The results of quality control of the dry extract of milk thistle according to the Russian Pharmacopoeia are presented, as the first stage for utilize the active phytosubstance silymarin in a complex hepatoprotective therapy.

Keywords: *Hepatoprotection, Combined preparation, Phytosubstances, Milk thistle extract.*

Nowadays, liver damage is widespread among the population. Increasing cases of liver diseases are associated with the continued growth of toxic, medicinal, viral and autoimmune effects on this organ, the high prevalence of metabolic disorders against the background of diabetes mellitus and obesity. Hepatoprotectors prevent the membrane destruction in the liver functional cells (hepatocytes), and stimulate their regeneration.

Studies on utilization of drugs containing silymarin, vitamin E, ursodeoxycholic acid, silibinin, glycyrrhizic acid and essential phospholipids in different combinations have shown that the simultaneous usage of hepatoprotective drugs and adjusted combinations can achieve a better clinical effect, as well as expand the indication ranges for individual drug uses [1].

The study of natural medicine has shown that extracts of some plants contain phytochemicals with hepatoprotective properties. Silymarin is one of these phytochemicals. It is extracted from dried seeds and fruits of milk thistle (*S. marianum*). Milk thistle extract (silymarin) is a complex mixture of plant-derived compounds identified mainly as flavonolignans, flavonoids (taxifolin, quercetin), and polyphenolic molecules [1].

Materials and methods. The object of this study was the extract of milk thistle fruits and seeds. In the study, the quality control of the extract was carried out, in accordance with the requirements of the Russian Pharmacopoeia XIV Ed. [2].

Results and discussion. As quality control results, it was determined that 90,85 % of the dry extract has a particle size of 1-2 mm or more, the humidity content is not more than 4%, the bulk density is 1,6786 g/cm³, the content of the total flavonolignans in the extract in terms of silybin is 17,7 %.

Conclusion. The results of the study, represent, in terms of quality, that the dry extract meets the requirements of the Russian Pharmacopoeia XIV Ed. The flavonolignans content of the extract in terms of silybin is 17,7 %. Thus, milk thistle dry extract is a promising phytochemical in the development of new combined preparations for the hepatoprotective therapy.

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Резюме

Для индивидуальной или комбинированной гепатопротекторной терапии, такие как экстракты расторопши, бессмертника, артишока, мяты и лабазники. Приведены результаты контроля качества сухого экстракта расторопши согласно Российской фармакопее как первого этапа использования активной фитосубстанции силимарина в комплексной гепатопротекторной терапии.

Ключевые слова: *гепатопротекция, комбинированный препарат, фитосубстанция, экстракт расторопши.*

DEVELOPMENT OF A COMBINED HEPATOPROTECTIVE PREPARATION

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Abstract. One of the broadest drugs groups used as part of complex therapy at different stages of liver damage are hepatoprotectors. In the present essay it was determined the dry extract Milk Thistle quality indicators. It was found that the Milk Thistle dry extract is a promising phytocomponent in the development of new formulations of combined hepatoprotective therapeutic drugs.

Keywords: *Liver illnesses, hepatoprotectors, milk thistle, dry extract, quality indicators, technological characteristics.*

Introduction. Today, liver damage is widespread among the population. They can either occur independently or accompanied by systemic pathologies. The increasing of cases liver diseases is associated with the continuous toxic growing, medicinal, viral and autoimmune effects on this organ, the high prevalence of metabolic disorders against the background of diabetes mellitus and obesity. One of the broadest drugs groups used as part of complex therapy at different stages of liver damage are hepatoprotectors. These are agents that prevent the destruction of the membranes of the functional cells of the organ (hepatocytes) and stimulate their regeneration. In the clinical practice, several hepatoprotective agents are often used simultaneously in form of individual drugs or combinations. Combined use can provide both an enhancement of a particular pharmacological effect and an expansion of the spectrum of hepatotropic action [1].

Aim of the Study. According to the current available hepatoprotectors, it seems promising to develop a new combined preparation containing total extracts from Milk Thistle in combination with Ursodeoxycholic acid.

Materials and Methods. The material of the study was Milk Thistle dry extract, manufactured by OOO LLC "Kazan Plant extracts" (Kazan, Russia). The dry extract standardization was achieved carrying out the quality indicators required by the Estate Russian Pharmacopoeia 14 ed. [2].

Results and discussion. Quality technological indicators such as: flowability, weight loss during drying ($0,79 \pm 0,04$ %), compressibility ($48,97 \pm 2,45$ N), bulk density ($1,6786 \pm 0,35$ g /cm³) were determined. The fractional composition essay, shown that the $71,03 \pm 3,55$ % of the dry extract have a particle with a size of 2-2,5 mm. As a result of the study, it was found that Milk Thistle dry extract has poor flowability, which must be taken into account during the drug-tech developing. As part of the of the quality specifications of the Milk Thistle dry extract, was carried out the flavolignans content determination, in terms of silybin, the dry extract contents $17,69 \pm 0,88$ %.

Conclusion. Thus, Milk Thistle dry extract is a promising phytocomponent in the development of new formulations of combined hepatoprotective therapeutic drugs.

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**THE NATURAL DEEP EUTECTIC SOLVENTS FOR EXTRACTION
OF DIFFERENT PARTS OF THE ROOT OF *ARALIA MANDSHURICA***

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Introduction. Triterpene saponins of *Aralia elata* var. *mandshurica* (Rupr. & Maxim.) J. Wen (1994) (*syn. A. mandshurica*) determines the influence on the metabolomic syndrome, cardiac system, and others [1]. The ethanol extract of plant has clinically proven an adaptogenic effect [2]. However, ethanol hurts the body in terms of pharmacology. The perspective alternative of these extractants and other toxic is the natural deep eutectic solvents (NADES). For the preferably, researchers Choi and co-workers discovered this type of solvent in 2011. Primary metabolites (sugars, organic acids, amino acids, etc.) contained in plant material are donors and acceptors of hydrogen bonds. When two or more components are fused, the melting point of the mixture decreases and a liquid is formed. This liquid can potentially be used as an extraction medium [3].

Aim of the Study. To study the structure and content of triterpene saponins that can pass from the internal environment of the cell to different parts of the root into NADES.

Materials and Methods. In our study we use extracts of core of the root and the root bark with water and NADES. The extracts were analyzed by the reversed phase-ultra-high-performance chromatography-mass spectrometry (RP-UHPLC-MS). Analysis based on earlier known composition of plant and used principles of metabolomic profiling for the determination of triterpene saponins. The relative content was counted with fold changes. Statistical processing was carried out using GraphPad Prism 9.

Results and Discussion. The twenty triterpene saponins were found in six NADES extracts of the core of the root and the root bark [4]. The quantity of twelve of them was higher in NADES in comparison to water. Figure 1 shows the structures and relative contents in NADES of two triterpene saponins (calendulglycoside C isomer 1 (a) and araloside A isomer 1 (b)) in comparison in aqueous extracts.

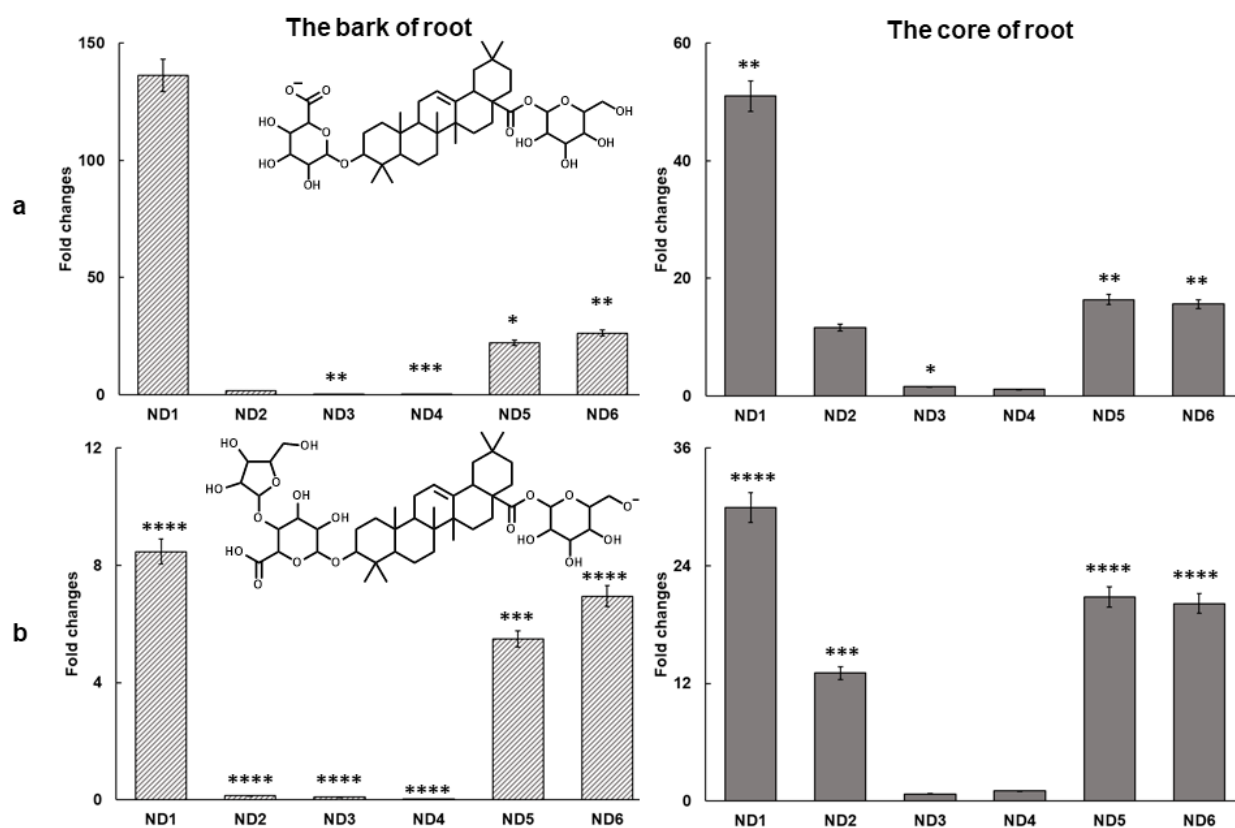


Figure 1. Structures and relative contents (in comparison in aqueous extracts) of calendulglycoside C isomer 1 (a)

and araloside A isomer 1 (b). *, **, ***, **** – statistically significant differences

($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$ respectively). ND1 – choline chloride–malic acid mixture (molar ratio 1:1).

ND2 – choline chloride and malic acid (1:2). ND3 – choline chloride and lactic acid (1:3). ND4 – choline chloride and lactic acid (1:3 + 30% (*v/v*) water). ND5 – sorbitol and malic acid (1:1 + 10% (*v/v*) water). ND6 – sorbitol and malic acid (1:2 + 20% (*v/v*) water)

As can be noticed these compounds were higher in ND1 (choline chloride and malic acid (1:1)) into 136, 51, 9, and 30 folds. Results point to that solvent has a greater affinity for these structures than water. Interestingly that the patterns of contents are similar which can be explained by resembling structures (only the presence of a pentose differs).

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OBTAINING GELS BASED ON AQUEOUS EXTRACTS OF YARROW

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Keywords: *yarrow herb, aqueous extracts, penetration ability, flavonoids.*

Introduction. Aqueous extracts of yarrow have antispasmodic, choleric, antioxidant and anti-inflammatory properties due to the flavonoids contained in yarrow herb. Thus, there is a need to study the penetrating ability of flavonoids from aqueous extracts for further realization of optimal therapeutic effect.

Aim of study. To study the penetration ability of flavonoids of yarrow herb on the model of gelatin gel based on the course of the chemical reaction with aluminum chloride and on the model of gelatin gel with the addition of lead acetate basic.

Materials and methods. The object of the study was yarrow herb produced by Biotest Research and Production Complex LLC, Republic of Belarus. To obtain an aqueous extract several methods of obtaining were used: in accordance with instructions for medical use (MU); according to generally accepted methods according to State Pharmacopoeia of Republic of Belarus (01/2013: RB0002 "#Infusions, decoctions and teas"); by the developed method, selected experimentally in earlier studies [1, 2, 3]. Preparation of decoctions was carried out in infundibles, and infusions at home on a water bath.

Results and discussion. The penetration rate of flavonoids from infusions and decoctions prepared in accordance with the instructions for MU, according to generally accepted methods according to State Pharmacopoeia of Republic of Belarus, as well as the developed technology at home in a water bath and in infundibles, after 36 hours using 2 % aqueous aluminum chloride solution was 9.7 mm. The penetration rate of flavonoids after 36 hours in the model with the addition of basic lead acetate was 3.5 mm. The degree of penetration of flavonoids from the infusion prepared according to the instructions for MU in home conditions in a water bath after 36 hours in the model with the addition of basic lead acetate was 3.7 mm.

Conclusions. Based on the above data, we can conclude that the gelatin gel model based on the reaction with aluminum chloride is optimal for studying the penetration ability of flavonoids of yarrow herb.

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**HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) METHOD
FOR QUANTIFICATION GINSENO SID RG1 IN THE SHENGM AI SAN**

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Keywords: *Shengmai San; Ginsenosid Rg1; High performance liquid chromatography.*

Introduction. Shengmai San (SMS), which is comprised of the medicinal herbs of Panax ginseng C.A. Meyer, Schisandra chinensis Baill., and Ophiopogon japonicus Ker-Gawl, is a traditional Chinese medicine can use for treating heat stroke and heat exhaustion, the serious clinical entities important in military, sports, occupational and civilian medicine.

Ginsenosides are known as the key active ingredient of ginseng, the main herbal medicines in SMS. Among them, ginsenoside Rg1 is one of the most abundant and usually regarded as the main ginsenosides. It has the many effect such as: Anti-depressant effect, anti-inflammatory effect, neuroprotective effect, protection against sepsis-associated encephalopathy [1].

Aim of the Study. In this study, we develop and valide a analysis procedure to quantitative Ginsenosid Rg1 in the SMS using high performance liquid chromatography (HPLC).

Materials and Methods:

Materials:

Reference Solution: Dissolve an exact amount of Ginsengnosid Rg1 (R) in methanol (R) and dilute to 10ml.

Test solution: Weigh accurately about 6,2g powder of SMS to a suitable flask, add 25ml methanol (R) and sonicate for 30 min, after cooling, filter though a suitable filter to collect the liquid; Treat the residue as described above. Mix the collected liquids and evaporate to suitable volume under reduced pressure at a temperature not exceeding 60°C; dilute to 25 ml if necessary. Before injection, pass through a filter of 0.45 µm pore size.

Methods:

Using HPLC with the following parameters:

Column: Pursuit C18, 250mm x 4,6mm x 5µm

Mobile phase: mobile phase A: water; mobile phase B: acetonitrile R with gradien:

- 0 to 10 minute: 80%A and 20%B;

- 10 to 20 minute: from 80% to 70% A and from 20% to 30% B;

- 20 to 24 minute: 70% A and 30% B;

- 24 to 30 minute: from 70% to 80% A and from 30% to 20% B.

Flow: 0.8 ml/min

Injection Vol: 20 µl.

Detection: spectrophotometer at 203 nm.

Validate this procedure according to ICH guidelines [2].

Results and discussion:

All the parameters: System suitable testing (with RSD of pic area and retention time <2%); Linearity (standard curve equation: $y = 8110,6x + 18785$ with $R^2 = 0,9998$); Specificity, Repeatability (RSD=0.87%), Accuracy (recovery: 95%-104%) were met ICH guideline for method validation.

Conclusions/ The quantitative analysis method of Ginsenosid Rg1 in the Shengmai San using HPLC has been developed and validated.

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OBTAINING TISSUE CULTURE OF *SALVIA OFFICINALIS* L.

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Introduction. Currently, there is an increasing interest in herbal medicines. Medicines based on *Salvia officinalis* L. (*Lamiaceae*) have anti-inflammatory, antitumor, hypoglycemic, neuroprotective, antimutagenic, antioxidant, antibacterial, lipid-lowering effect. Plant contains essential oils, tannins, flavonoids, hydroxycoric acids, phenolic compounds [1].

Aim of study. The aim of the study is to obtain a viable callus culture of *Salvia officinalis* L.

Materials and methods. Phytobiotechnologies methods are used for work. Leaves of an intact plant sage medicinal, of the *Lamiaceae* family (*Salvia officinalis*, *Lamiaceae*) were used as explants. The explants were pre-sterilized with 6 % sodium hypochlorite solution for 20 minutes and 70 % ethanol for 1 minute. It was cultivated on a nutrient medium according to the Murasig – Skoog recipe. The medium contained phytohormones: 2,4-dichlorophenoxyacetic acid (6 mg / ml) and kinetin (1 mg / ml) and half concentration of micro and macro salts. Callus was cultivated in the dark, temperature 27-28°C, humidity 60 – 70%.

Results and discussion. Formation of the primary callus on the leaf surface was observed after two weeks of cultivation. After the second passage, the strains transplanted to MS with a complete content of micro and macro salts and phytohormones: 1-naphthylacetic acid (1 mg/ml) and kinetin (1 mg/ml). The color of the cells changed from dark grey to light grey. A study of growth activity was performed at the seventh passage. The detected cells during microscopy can be divided into two types: the first type is cells of the meristematic type, the second type is cells of the parenchymal type. Microscopy showed that more than 95 % of all visualized cells are alive. Strain growth is characterized by a standard "S" – shaped curve (Sachs curve). Qualitative analysis carried out for dry biomass. Qualitative reactions showed the presence of tannins, flavonoids and phenolic glycosides.

Conclusions. Callus culture of plant cells *Salvia officinalis* L. (*Lamiaceae*) was obtained. Growth characteristics of the strain were studied. Qualitative analysis for the main functional groups was carried out. Further studies are aimed at determining the qualitative and quantitative composition of biologically active substances.

Funding. The results of the work were obtained using the equipment of the Central Collective Use Center "Analytical Center of the Federal State Budgetary Educational Institution of Higher Education SPCPU of the Ministry of Health of Russia" under agreement No. 075-15-2021-685 dated July 26, 2021 with financial support from the Ministry of Education and Science of Russia.

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THE CONTENT OF COUMARINS AND TANNINS IN *HAPLOPHYLLUM DAURICUM* (L.) G.DON

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Keywords: *Haplophyllum dauricum*, coumarins, tannins.

Introduction. Today there is an increased interest in the search and study of plants as a source of antitumor substances. *Haplophyllum dauricum* has been widely used in Mongolian folk and Tibetan traditional medicine for tumor treatments [1]. Lignans and coumarins are the main active compounds. The chemical composition of *H. dauricum* of Russian flora has not been studied before. In previous studies we analyzed the composition of essential oils and fatty acids; also, we isolated and established the structures of arylnaphthalene lignans which showed cytotoxic activity against tumor cells [2-3].

Aim of study. The aim of this study was to determine the total content of tannins and coumarins in *H. dauricum*.

Materials and methods. Herb and roots of *H. dauricum* were collected in the flowering and fruiting phase in 2020-2021 in three districts of the Republic of Buryatia and in the Trans-Baikal Territory. The total content of tannins was determined by redox titration (permanganometry) (in terms of tannin) and by UV spectroscopy (in terms of gallic acid). The content of the total coumarins was determined by the spectrophotometric method (in terms of scopoletin) using a calibration curve, after extraction of the raw material by ultrasound-assisted extraction with 80% methanol. A total of 4 samples of herb and roots of *H. dauricum* were studied. The study was conducted in five repetitions, and the measurement error did not exceed 5%.

Results of discussion. This study was the first to investigate the total content of coumarins and tannins in the herb and roots of *H. dauricum* growing in Trans-baikal Territory. The total content of tannins in the roots was 2.69-3.13%, while in the herb it was 6.31-11.20%. The lowest content of tannins was found in the roots collected in the Aginsky district of the Trans-Baikal Territory (0.87%). In contrast, the samples from Selenginsky district of the Republic of Buryatia had the highest content (1.35%). In herbs collected within the territories of the Khorinsky and Dzhida districts the contents were a bit higher – from 1.01 to 3.31%, respectively. The content of coumarins in the roots varied from 1.84 to 2.77% (Dzhidinsky and Selenginsky districts), and in the herb – from 4.32 to 7.09% (Selenginsky and Khorinsky districts).

Conclusion. Thus, *H. dauricum* is a rich source of polyphenolic compounds. It was found that roots contain a high concentration of coumarins that reaches 2.77%, and herb – up to 7%. In addition, plants from Trans-Baikal accumulate higher concentration of substances than that from Mongolia.

Funding. This study was funded by the framework of the State Assignment of BINM SB RAS (Project No. FWSU-2021-0010) on the ISEC topic “Baikal” using the resources of CCU, BINM SB RAS.

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**IDENTIFICATION AND QUANTITATIVE DETERMINATION OF FLAVONOIDS
BY HPLC-UV METHOD IN THE RAW MATERIALS OF SOME REPRESENTATIVES
OF THE GENUS *RUMEX* OF THREE VEGETATION TIMES**

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Keywords: HPLC UV, *Rumex*, flavonoids, metabolomics, phytochemistry.

Introduction. The study of the accumulation of substances of a phenolic nature in medicinal plant materials is an important issue in the study of the metabolomic composition. [1,2]

Aim of study. Identify and quantify 8 aglycone and glycosides of flavonoids in 4 species of the genus *Rumex* in 3 different vegetations. To reveal the pattern of accumulation of phenolic compounds depending on the phase of vegetation for different types of *Rumex*.

Materials and methods. The content of biologically active substances was determined by high performance liquid chromatography with ultraviolet detection. Detection was carried out at a flavonoid-selective wavelength of 365 nm, a mixture was used as the mobile phase and gradient elution with a 0.1% solution of formic acid in water (by volume) (eluent A); 0.1% solution of formic acid in acetonitrile (by volume) (eluent B), Grace HPLC-COLUMN 250x4.6mm platinum C18-EPS 5mm chromatographic column (Grace, USA).

Results and discussion. As a result of studies in the underground organs, the following content of the sum of some flavonoids was established: quercetin-3-O-rutinoside (rutin), 3-O-rutinoside of isorhamnetin (narcissin), luteolin, isorhamnetin, 7-O-beta-D-glucoside apigenin (kosmosiin), 3-O-glucoside of kaempferol (astragalin), kaempferol, 7-O-glucoside of luteolin (cynaroside) The substances were analyzed by HPLC-UV, the total content was: in the phase of spring regrowth (SR) in *R.confertus* – 0.431 ± 0.026%, *R.aquaticus* – 0.433 ± 0.035%, *R.crispus* – 0.121 ± 0.011%, *R.obtusifolius* – 0.194 ± 0.013%; in the flowering (F) phase in *R.confertus* – 0.341 ± 0.014%, *R.aquaticus* – 1.008 ± 0.41%, *R.crispus* – 0.353 ± 0.036%, *R.obtusifolius* – 0.443 ± 0.023%; in the phase of overhead part dieback (OPD) in *R.confertus* – 0.066 ± 0.005%, *R.aquaticus* – 0.211 ± 0.017%, *R.crispus* – 0.035 ± 0.002%, *R.obtusifolius* – 0.051 ± 0.007%.

The content of 3-O-glucoside kaempferol (astragalin) is the highest in *R.crispus* in the SR phase – 0.026%, in the *R.confertus* was not found in any growing season, kaempferol is the highest in *R.aquaticus* in the F phase, in the *R.confertus* and in *R.crispus* was not found in season OPD, quercetin-3-O-rutinoside (rutin) is the highest in *R.aquaticus* in SR phase, in *R.confertus* and in *R. obtusifolius* was not found in any growing season, luteolin is the highest in *R. aquaticus* in the F phase and the lowest in *R. obtusifolius* in OPD phase, 3-O-rutinoside of isorhamnetin (narcissin) is the highest in *R. aquaticus* in the F phase and the lowest in *R. confertus* in SR phase, isorhamnetin is the highest in *R. aquaticus* in the SR phase and the lowest in *R.crispus* in OPD phase. 7-O-beta-D-glucoside apigenin (kosmosiin) and 7-O-glucoside of luteolin (cynaroside) were not founded in all objects.

Conclusions. In the underground organs of four representatives of the genus *Rumex*, the content of flavonoid aglycones and glycosides for 3 species is higher in F phase and decreases during OPD and SR phase. The exception is *R.confertus*, the content of aglycone and glycosides flavonoids in this species is higher in the SR phase. The highest content is observed in *R.aquaticus* in the flowering phase – 1.008 ± 0.041%. *R.crispus* has the least – 0.035 ± 0.002%.

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EXPERIENCE OF THE INTRODUCTION OF SWEET BASIL IN CAUCASIAN MINERAL WATERS

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The work is devoted to the introduction of sweet basil in the region of Caucasian Mineral Waters and the subsequent determination of essential oil in its herb.

Keywords: *sweet basil, introduction, essential oil.*

Introduction. Sweet basil (*Ocimum basilicum* L.), the family Lamiaceae, is an annual herbaceous essential oil plant, the culture of which originates from North Africa since ancient times [1]. For the cultivation of basil in temperate countries there are essential the duration of the warm season and the sum of effective summer temperatures [2, 3]. The region of the Caucasian Mineral Waters meets the climatic requirements for growing this plant.

Aim of study. To conduct introduction studies of the possibility of growing sweet basil and its ability to accumulate essential oil in open ground conditions of the Caucasian Mineral Waters.

Materials and methods. The seeds of sweet basil of two varietal samples: purple and green, agricultural firm "Gavrish", Russia. To determine the essential oil content the method of hydro distillation was used (State Pharmacopoeia Russian Federation 14th edition, Vol. 2, 2018).

Results and discussion. Sowing was carried out in open, well-lit experimental plots of 1×1 m (1 sq. m), laid in the vicinity of Pyatigorsk, Stavropol Krai, in April 2022, when the average minimum temperatures exceeded 5 °C, and the average maximum 12 °C. The phenological phases of basil are given in the Table.

Table – Phenological phases of sweet basil introduced in the Caucasian Mineral Waters

Phenological phase	Emergence of seedlings	Growth of shoots	Бутонизация	Mass flowering	Fruiting
Dates	May 10–15	June 10–30	July 6–15	July 20–30	August 16 to early September

It should be noted that the phenological phases of both varietal samples completely coincided, which indicates their biological identity. The collection of raw materials – the herb of sweet basil – for the quantitative determination of essential oil was carried out in the last decade of July, in the phase of mass flowering, when more than 80% of the flowers on all specimens were fully bloomed. The essential oil content in the purple varietal sample was $0.82 \pm 0.04\%$, in the green varietal sample was $0.84 \pm 0.05\%$.

Conclusions. In the conditions of the Caucasian Mineral Waters, the sweet basil has a late vegetation, which should be taken into account when growing. The content of essential oil of both varietal forms is comparable with the content of this indicator for many essential oil types of medicinal plant raw materials.

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**COLLECTIONS OF PLANT CELL CULTURES AS A BASIS
FOR BIOTECHNOLOGICAL PRODUCTION OF PHYTOPREPARATIONS****Popova Elena¹, Titova Maria¹, Nosov Alexander^{1,2}**¹ K.A. Timiryazev Institute of Plant Physiology of Russian Academy of Sciences
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Human life largely depends on the medicines provided by wild plants, but their diversity is depleting rapidly. Recent studies estimated that about 15,000 medicinal plant species are threatened with extinction from overharvesting and habitat destruction. Sustainable production of novel food and plant-derived bioactive components through biotechnological approaches such as cell farming could eliminate or significantly reduce the industries' dependence on wild plant resources. To be economically effective, these technologies require a large genetic base of the cell culture strains with high productivity of the desired bioactive compounds.

The All-Russian Collection of Plant Cell Cultures hosted by the K.A. Timiryazev Institute of Plant Physiology of the Russian Academy of Sciences is the oldest and most diverse Russian collection of plant cell cultures. The first cell cultures of *Panax ginseng*, *Rauwolfia serpentina*, *Catharanthus roseus*, *Dioscorea deltoidea*, and other medicinal plants were developed by Prof. Butenko and her team in the 1950s–60s; some of those cultures are still maintained in the active collection. Currently, the collection holds 43 cell culture strains of 24 plant species as the core collection; 74 strains of 32 plant species are maintained for experimental purposes. Historically, the collection has been focused on cell cultures accumulating isoprenoid compounds (furostanol glycosides, ginsenosides, taxoids, etc.). The most promising, from the pharmaceutical viewpoint, are cell strains of *Dioscorea deltoidea* with total content of 25(S)-protodioscin, protodioscin, and deltoside up to 4.6–5.7% of the dry cell weight (DW), *Panax ginseng*, and *P. japonicus* cell strains (ginsenoside and their derivatives up to 3.46% DW), *Tribulus terrestris* (total content of furostanol glycosides 0.1% DW), *Polyscias filicifolia* and *P. fruticosa* (total content of polysciosides and their derivatives 0.5–3% DW). The experimental collection contains cell strains at different stages of growth optimization and biochemical evaluation, e.g. cell cultures of medicinal plants *Sutherlandia frutescens*, *Ajuga turkestanica*, *Alhagi persarum*, *Maackia amurensis*, *Cladochaeta candidissima*, and *Panax vietnamensis*. Stably growing cell strains with high content of the desired metabolites are further subjected to cultivation in a cascade of bioreactors (20-L – 75-L – 630-L). Large-scale (630-L) bioreactor production has been developed and routinely applied for suspension cell cultures of *Dioscorea deltoidea*, *Polyscias filicifolia*, *Panax japonicus*, and *Taxus wallichiana*. Smaller bioreactors (up to 75-L) were successfully tested for *Tribulus terrestris*, *Taxus baccata*, *Polyscias fruticosa*, *Panax vietnamensis*, *Stephania glabra* and some other strains.

Evaluation of biological activities of bioreactor-produced cell biomass is the next step towards its certification. Phytopreparations based on cell cultures of *Dioscorea deltoidea*, *Panax japonicus*, and *Tribulus terrestris* were shown to exhibit a range of positive effects in rats with induced type 2 diabetes mellitus and obesity. These included reduced daily diuresis, blood-glucose and total cholesterol levels, reduced weight gain and normalization of body fluids balance. The safety of *D. deltoidea* cell biomass was confirmed by toxicological assay and elemental composition analysis. Several commercial products containing bioreactor-produced cell biomass are currently available in the market. Food additive Vitagmal © is based on dried biomass of *Polyscias filicifolia* cell culture which had passed the clinical trial with proven adaptogenic and anti-teratogenic effects. Cell culture of *Panax ginseng*, strain G1, is currently used for producing a series of cosmetics and food additives (<https://cosmevita.ru/collections/>).

In conclusion, the All-Russian Collection of Plant Cell Cultures holds a valuable genepool of strains for biotechnological application that are a promising source of plant bioactive compounds for pharmacology, cosmetic and health products.

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ANTHOCYANINS OF THE FRUITS OF STAGHORN SUMAC INTRODUCED IN THE STAVROPOL KRAI

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Keywords: *staghorn sumac, introduction, anthocyanins.*

Introduction. Staghorn sumac (*Rhus typhina* L.), the family Anacardiaceae is one of the few representatives of the sumac genus that can be introduced in temperate regions due to good winter hardiness [1, 2]. Every year one tree produces a large biomass of fruits [3]. However the fruits of staghorn sumac have not been studied phytochemically in our country.

Aim of study. Study of anthocyanins of the fruits of staghorn sumac introduced in the Stavropol Krai.

Materials and methods. Dry fruits of staghorn sumac harvested in September 2022 during the ripening phase of the fruits of plants introduced in the botanical garden of the Pyatigorsk Medical and Pharmaceutical Institute (PMPI) and in the vicinity of Pyatigorsk, Zheleznovodsk, Georgievsk – in total 5 samples of raw materials. The determination of anthocyanins was carried out according to the method of the State Pharmacopoeia of the Russian Federation 14th edition, proposed for the fruits of dry black chokeberry [4].

Results and discussion. Anthocyanins were found in the fruits of staghorn sumac by thin-layer chromatography, among which the dominant component is cyanidin-3-O-glycoside. The quantitative content of anthocyanins in five samples of the fruits of staghorn sumac in terms of absolutely dry raw materials is given in the table.

Table – Quantitative content of anthocyanins of the fruits of staghorn sumac

Sample	Botanical garden of the PMPI	Pyatigorsk, city center	Pyatigorsk, a suburb	Zheleznovodsk, city park	Georgievsk, infield
Content of anthocyanins	4.14 ± 0.04	4.16 ± 0.05	4.22 ± 0.05	4.20 ± 0.04	4.19 ± 0.05

It can be seen from the table data that the content of anthocyanins in the fruits of staghorn sumac, introduced in various parts of the Stavropol Krai, does not differ in variability and averages $4.18 \pm 0.05\%$.

Conclusions. Staghorn sumac is a deciduous tree, hardy, widely cultivated in the Stavropol Krai. The presence of anthocyanins in the fruits of staghorn sumac, as well as their quantitative content, allows us to characterize this raw material as a promising source of biologically active compounds.

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PROCOGNITIVE EFFECT OF *CISTUS SALVIIFOLIUS* L. IN EXPERIMENTAL STEATOHEPATITIS

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Effects of a Sage-leaved rock-rose *Cistus salvifolius* L. (CS) aqueous extract were assessed using the Spontaneous alternation in the T-maze and tests in adult male C57Bl/6 mice with a diet/chemical-induced model of NASH. CS extract increased the short-term spatial memory but did not affect short-term recognition memory of the animals, indicative of its potential procognitive action in metabolic disease.

Keywords: *Cistus salvifolius*, spatial memory, cognitive deficits, non-alcoholic steatohepatitis

Introduction. Non-alcoholic steatohepatitis (NASH) is a highly incident and prevalent chronic liver disease with multiple extrahepatic, including neuropsychiatric, complications and comorbidities. Sage-leaved rock-rose *Cistus salvifolius* L. (CS) is a flavonoid and polyphenol-rich plant with a wide spectrum of biological activities, including possible procognitive effects.

Aim of the study. The present study was aimed at exploring the potential procognitive effects of a CS aqueous extract in memory dysfunction associated with murine alimentary/toxic NASH.

Materials and methods. NASH was induced in 60 young adult male C57Bl/6 mice randomized by 30 into the following groups: (1) Control: NASH + no treatment; (2) CS: NASH + 253 mg·kg⁻¹ b.w. CS aqueous extract (equivalent to human daily therapeutic dose). NASH was induced over 3 months [1], and the drugs were administered orally q.d. during the entire experimental period. Short-term memory was assessed using the Spontaneous alternation in the T-maze (SATM) and Novel object recognition (NOR) tests according to conventional protocols.

Results and discussion. In the SATM test, the CS extract increased the spontaneous alternation rate by 126% compared to Control ($p < 0.05$), which indicates a marked improvement in short-term spatial memory. In the NOR test, the extract had no effect on object exploration and short-term recognition memory (Figure). Allocentric spatial memory is supposed to require more hippocampal tissue [2]; therefore, the selectivity of CS's effect might be explained by the distinct nature of the two neural systems respectively involved in object and spatial recognition.

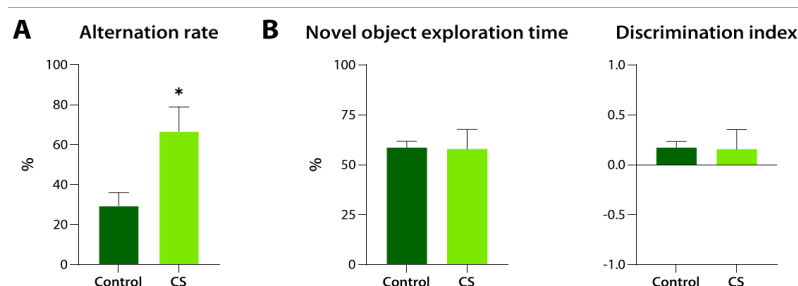


Figure. Animal short-term memory as assessed by the Spontaneous alternation (A) and Novel object recognition (B) tests. * $p < 0.05$

Conclusions. A CS aqueous extract improved short-term spatial memory in experimental NASH, which indicates potential procognitive activity of CS in metabolic disease.

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OPTIMIZATION OF *PAEONIA ANOMALA* L. EMBRYOCULTURE TECHNOLOGYRaizer Olessya¹, Tagimanova Damelya¹, Khapilina Oksana¹¹National Center for Biotechnology

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Introduction. *Paeonia anomala* L. is a valuable medicinal plant listed in the Red Book of Kazakhstan. Renewal of the species in natural conditions and reproduction in introduction culture is difficult due to the slow germination of seeds. Seeds of *P. anomala* have an underdeveloped embryo, which is characterized by a very low content of physiologically active substances and weak enzyme activity [1]. Cultivation of isolated tissues and *in vitro* organs is optimal for hard to renew species, as well as to preserve their gene pool. One of the first stages of *in vitro* cultivation is to obtain sterile and viable explants, so the development of sterilization and embryoculture methods ensures the further success of microclonal multiplication technology.

Aim of study. Elaboration of optimal method of seed sterilization of *P. anomala* L. when introduced into culture *in vitro* for preservation and reproduction of this species biodiversity.

Materials and methods. Seeds of wild-growing plants of *P. anomala* L. were used as an object of research. Seeds after preliminary sterilization with different types of antiseptics were placed on nutrient media [2]. Concentrations and exposure time for each sterilizing agent were chosen empirically. After sterilization, seeds were placed on nutrient medium ½ MS with the addition of 5 mg/L GA3 and 0.1 mg/L IUC, and cultured at 25°C, a time of warm stratification. After 21 days, sterile and swollen seeds of *P. anomala* L. were used for *in vitro* culture. Germs 1-2 mm were isolated from the seeds and placed on nutrient medium ½ MS supplemented with 0.5 mg/L BAP and 0.5 mg/L GA3.

Results and discussion. Seven multistage schemes of seed sterilization, consisting of pre-sterilization, soaking, sterilization itself, were worked out. The optimal protocol was that of washing in flowing water, incubation in nystatin solution (5000 units/ml) followed by treatment in 0.1% mertiolate solution, exposure time 20 minutes. After 10-14 days, 30-50% of the embryos showed yellow-white seedling proliferation, and anthocyanin pigmentation appeared on some explants, indicating successful germ development. To increase proliferation and remove the deep epicotyl dormancy characteristic of *Paeonia* germs, we first suggested using 2-fold increased concentrations of CaCl₂, by analogy with the *Paeonia lactiflora* [3]. This helped to increase the number of viable normally developed embryos to 75%...85%, and in general, increased the efficiency of embryoculture method.

Conclusions. The results showed that the selected scheme of seed sterilization and cultivation conditions of isolated embryos of *P. anomala* L. allowed to obtain a sufficiently high percentage of viable explants for subsequent microcloning under *in vitro* conditions.

Funding. The work was carried out under the program-targeted funding BR18574125.

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ELEMENTAL ANALYSIS OF NEEM LEAVES TO ASSESS ITS USE SAFETY

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Abstract. Abroad, a risk-based strategy is used to assess the safety of using native products. In the study was analyzed and compared the risk to human health with long-term use of neem leaves and its native powder in terms of heavy metal content.

Keywords: *neem, leaves, safety, risk of use, heavy metals.*

Introduction. *Azadirachta indica* A. Juss/Neem tree/Margosa Tree leaves (Fam. *Meliaceae*) have a wide range of pharmacological effects: anti-inflammatory, antibacterial, antifungal, immunomodulatory, antioxidant. It is widely used in Chinese and Ayurvedic medicine for various diseases treatment.

Aim of study. To assess the mineral composition and possible elements intake into the human body using the risk-based strategy when taking Neem raw materials.

Materials and methods. The study objects were whole leaves of Margosa Tree (Hasan, India) and fine neem powder sold on the Russian pharmaceutical market. The elemental composition study included 2 stages: sample preparation and instrumental analysis. Sample preparation was conducted by "wet" mineralization in the microwave decomposition system Speedwave two (Berghof, Germany), after that the 22 elements content was studied (*Al, As, Ba, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Zn*) on the atomic emission spectrometer Optima 8000 ICP-OES (PerkinElmer, USA).

Results and discussion. 13 elements (*Al, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Rb, Sr* и *Zn*) were determined in Neem leaves (Hasan, India) in the range of 0.84–20050 µg/g, and in powder – 3.33-13080 µg/g. Toxic elements *Pb, Cd* and *As*, as well as *Bi, Ni, Cs, Se* were not found in the samples. It has been established the *Ca, Cu, Fe, K, Mg, Mn, Na, Zn* admission in a 5-10 g daily dose provided the necessary these elements daily intake by 0.05-20.05% with the use of leaves, by 1.02-13.08% – with native powder. The risk assessment for combined exposure to heavy metals was based on the total hazard index calculation [1], which did not exceed 1 (0.3 and 0.2, respectively).

Table 1 – Metals hazard quotients for neem leaves and powder.

Element	Neem (Hasan, India)		Native neem powder	
	The study result, µg/g	The hazard quotients	The study result, µg/g	The hazard quotients
<i>Al</i>	174,6	0,03	133,7	0,02
<i>Ba</i>	67,8	0,16	23,4	0,06
<i>Cu</i>	156,7	0,09	104,8	0,06
<i>Fe</i>	22,67	0,03	11,79	0,02
<i>Mn</i>	0,84	0,007	3,33	0,03
<i>Zn</i>	10,32	0,006	15,37	0,008

Conclusions. There is no risk of adverse effects on human health with long-term use of neem (<1), and the intake of leaves satisfies the daily need for *Ca, Fe, Mg, Mn, Na* 1-7% more than native powder, which in turn, to a greater extent (2-4 times) meets the needs for *Cu, K, Zn*.

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**CHEMICAL CHARACTERIZATION OF *COCOS NUCIFERA* L. (COCONUT)
AND *BORASSUS FLABELLIFER* L. (PALMYRA) TODDY****Ranasinghe P.¹, Abeysinghe Ch.¹, Samarasinghe K.¹, Weeratunge H.¹**¹Modern Research and Development Complex, Industrial Technology Institute
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Introduction. Palm sap, which is collected from cut surface of immature inflorescence of palms is allowed natural fermentation by wild microflora consist of bacteria and yeasts, the resulting fresh beverage is called toddy or palm wine. The consumption of toddy is date back to very ancient ages and it is considered as a healthy natural beverage with mild levels of alcohol. In Sri Lanka, coconut and palmyra are popular toddy types which are traditionally prepared fresh and consume within the same day due to short shelf life. However, with market oriented commercial approaches, coconut and palmyra toddy are also currently manufactured as industrial products and there is raising need for setting up of standards for coconut and palmyra toddy to ensure the quality and authenticity.

Aim of the study. This study was focused to characterize the chemical properties including volatiles, alcohol residual sugars and polyphenolic contents of coconut and palmyra toddy.

Materials and Methods. Toddy samples (>15 from each type) were collected from coconut toddy tapping site in southern area and palmyra tapping sites in Northern area of Sri Lanka. All samples were centrifuged at 6000 rpm and filtered with 0.45-micron syringe filters prior to use analysis. HPLC was carried out in Agilent 1260 infinity II with Metacarb 67C column coupled with RI detector. To quantify ethanol, methanol and residual sugars. Soluble solid content and pH were measured, and total phenolic content was determined using folin-ciocalute method. Volatile profile of n-hexane fraction of toddy samples was analysed using GC- MS (Thermo Scientific Trace 1300) system with fused silica capillary column (Agilent DB-wax) and compound identification with NIST11 and FFNSC3 libraries.

All mass spectra compound data were imported to Rstudio and compounds with RSI (>790) were selected for analysis and summarization. All other data were subjected to two tail t test and presented as mean with standard error.

Results and Discussion. Soluble solid content and pH were found 4.5 ± 0.2 & 4.1 ± 0.3 and 3.4 ± 0.2 & 3.3 ± 0.1 respectively for coconut and palmyra. Quantitative results showed that coconut and palmyra toddy contain ethanol (0.56 ± 0.04 mg/ml, 0.35 ± 0.03 mg/ml), sucrose (104.79 ± 12.22 mg/ml, 123.14 ± 28.28 mg/ml), fructose (5.42 ± 0.83 mg/ml, 6.06 ± 0.91 mg/ml) and TPC (59.71 ± 8.74 μ g GAE/ml, 69.39 ± 19.94 μ g GAE/ml) respectively, glucose content was found to be below the limit of quantification and methanol was not detected. None of the parameters except ethanol content showed significantly difference between coconut and palmyra ($P=0.05$).

Analysis of volatile compounds showed overall 50 compounds from all samples but only 16 compounds were detected in more than 3 samples. No significant clustering of these 50 compounds between toddy type was found.

Results of this study demonstrate that basic chemical properties including volatile profile of coconut and palmyra toddy do not significantly different and may not be possible to use to distinguish two types.

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**FATTY ACIDS PROFILE OF YELLOW AND ORIENTAL MUSTARD SEEDS
AFTER GROWING ON SUBSTRATE WITH ZINC EXCESS****Repkina N.S.^{1*}, Murzina S.A.¹, Voronin V.P.¹, Kaznina N.M.¹**¹Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences (IB KarRC RAS)
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Introduction. Periodic adding in high amount manure into agriculturally used areas resulted in increase in microelements concentration [1]. The overconcentration of microelements caused a negative effect on quality and quantity of crop yield. Plants with high phytoremediation potential, such as *Sinapis alba* L. (yellow mustard) and *Brassica juncea* L. (oriental mustard) are used for decontamination soils from heavy metals excess. Also, both species widely used in food production [2].

Aim of the study. In present study the fatty acid profile and quality of seeds of *S. alba* and *B. juncea*, growing on substrate with zinc (Zn) excess were investigated.

Materials and Methods. The experiment was performed in greenhouse under natural conditions. Two-day-old seedlings (12 seedlings per a pot) of yellow and oriental mustard were grown on sand. Appropriate amounts of sulphate salt of Zn were added once to the sand to maintain the required levels of Zn: 5 (control), 50, 100, and 150 mg/kg of substrate. Pots were irrigated by Hoagland–Arnon nutrient solution excluding the addition of Zn. The experimental design was completely randomized with three replications. An inductively coupled plasma-mass spectrometry (ICP-MS) (Agilent 7900, Santa Clara, CA, USA) analysis was performed to determine the trace nutrients concentration in seeds. The fatty acid (FA) profile of the TL was analyzed through gas-liquid chromatography (GC) with mass-selective detector (MS).

Results and discussion. Along with increase in concentration of Zn in substrate its concentration was increase in seeds both *S. alba* and *B. juncea* seedlings. At the height Zn concentration (150 mg/kg) in substrate, the Zn concentration in seeds both species was about 4-fold increase in comparison to control. Except for Zn concentration in seeds both species and copper concentration in seeds of oriental mustard from plants growing at 100 and 150 mg/kg of Zn, all seeds are compliant with standards of SanPiN. The Zn and Cu are essential elements for human metabolism that's why these seeds can be used in food production for alleviate the deficiency of these nutrients in dietary. Both studied species, growing on contaminated substrate, contain higher amount of FAs. The increase of Zn concentration accompanied with the lifted FA content in seed of yellow mustard in comparison to the control. The highest total FAs content was due to increase in saturated FA (SFA). Despite the elevated SFA content, the ratio of unsaturated FA (USFA) to SFA was higher. The content of FAs in seeds of oriental mustard slightly decreased under all concentration of Zn in comparison to control. But in this case the proportion of USFA to SFA was also higher. The higher content of USFA is valuable for food production and human health.

Conclusion. The seeds of oriental and yellow mustard plants, growing on substrate with Zn excess can be used for food production for alleviate the deficiency of Zn and Cu in dietary and as source of USFA.

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PHENOLIC COMPOUNDS OF *BIDENS TRIPARTITA* HERB OF RUSSIAN ORIGIN

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Key words: *Bidens tripartita* herb, flavonoids, hydroxycinnamic acids, UHPLC-MS method, standardization.

Introduction. *Bidens tripartita* herb is a pharmacopoeial medicinal plant raw material. It is widely used in clinical practice to treat and prevent many common diseases. Despite the high degree of scientific knowledge of this medicinal raw material, the improvement of approaches to standardization remains an urgent issue [1, 2].

Aim of the Study. In this study, our goal was to study the composition and content of phenolic biologically active compounds (BAC) of the *Bidens tripartita* herb, growing on the territory of the Russian Federation.

Materials and Methods. For the analysis of phenolic compounds in industrial samples of *Bidens tripartita* herb, the UPLC method with photodiode array and MS/MS detection was used. Extraction was carried out with 70% aqueous methanol in an ultrasonic bath. To assess the content of flavonoids (SP, “total flavonoids contents in terms of rutin”), the methods described in the PM SP RF XIV edition for the *Bidens tripartita* herb were used.

Results and discussion. The presence of the phenolic compounds characteristic for *Bidens tripartita* herb was confirmed. We have identified 13 compounds, including hydroxycinnamic acids (isocaphtharic, caftaric, 3,5-O-dicaffeoylquinic, chlorogenic acids) and flavonoids (luteoline, luteoline-7-O-glucoside, sulfuretin 6-methylglucoside, sulfuretin 6-glucoside, okanine-4'-acetylglucoside, okanine-4'-glucoside, hypolaetin-8-glucoside, 2',3',3'-trihydroxy-4-methoxy-4'-acetylglucoside chalcone, quercetagenin-3-O-glucoside). The total flavonoids content was 0.7-1.2% (gravimetry). Rutin (absorption maximum at 415 nm) was confirmed as a standard (according to experimental UV spectra) for all samples.

Conclusions. The results obtained during the experiment are consistent with the literature data. When standardizing a medicinal herbal preparation based on the herb, it is recommended to use the following compounds as markers: sulfuretin and okaniin glycosides and the profile of hydroxycinnamic acids, as a specific group of BAC.

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**NIGELLA SATIVA L. IS A UNIQUE SOURCE
OF POLYPEPTIDES WITH PHARMACOLOGICAL PROPERTIES**

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It is well known that plants are an inexhaustible source of biologically active compounds with various types of functional action. Many of these compounds, mainly from among the secondary metabolites, their use in herbal medicine in the format of the so-called "traditional medicine". Many of these plant species are widely known and used as antipyretic, anti-inflammatory, antiseptic and antimicrobial agents. The latter group has recently acquired particular relevance due to the spread of pathogens of dangerous human infectious diseases of a bacterial and viral nature, as well as a decrease in therapeutic efficacy against the background of an increase in microbial resistance to antibiotics. Peptides from plants are an extremely attractive group of compounds for research, since in a number of cases they have almost the entire spectrum of biological properties as secondary metabolites, while, as a rule, to achieve the desired effect, lower effective concentrations are required, leading to a more prolonged action.

Nigella, or black cumin (*N. sativa*) is a unique plant widely used as a component of traditional medicine in the countries of the Middle East, Central and South-Eastern Asia. The spectrum of secondary metabolites of the seeds of this plant has been studied very well, however, the available knowledge on the structural and functional diversity of biologically active proteins and peptides is extremely limited to date. In a series of scientific studies to identify the spectrum of such molecules, compounds with pronounced antibacterial and antifungal properties [1–3], antiproliferative [2, 3] and anti-inflammatory activity, as well as efficacy against DNA and RNA viruses were identified. Thus, the obtained results provide a basis for further research of target individual seed polypeptides from *N. sativa* as pharmacological agents.

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PROSPECTS FOR THE PRODUCTION OF DOMESTIC PLANT-BASED GELLING AGENTS

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Introduction: As per latest market analysis on natural gelling agents by Fact.MR, a market research and competitive intelligence provider, the global market is projected to be valued at US\$ 3.27 billion in 2022, and expand a CAGR of around 6.8% over the period of 2022-2032. On the basis of product, the Gelling Agents Market is segmented into Agar-Agar, Gellan Gum, Curdlan, Xanthan Gum, Karaya Gum, Gelatin, Pectin, Guar Gum, Gum Arabic [1]. The special role of gelling agents is explained by the extreme range of their application. These natural and biodegradable extracted components are free of chemical reactions, giving them a competitive advantage in the market. Furthermore, natural gelling agents have a wide range of applications, including food and beverage and personal care, in regenerative medicine and tissue engineering. The major players operating in the Gelling Agents Market in the world are: USA, China, France, UK, Japan, Austria, Netherland, Hong Kong, India.

Aim of study: In this study, we assessed the global market for gelling agents and identified promising opportunities for domestic enterprises in the production of plant-based gelling agents.

Materials and methods: The main method of data collection is document monitoring. Traditional (qualitative) content analysis of interviews and documents was used as the main method of analysis, which was carried out as part of desk research.

Results and discussion: The demand for gelling agents on the Russian market is extremely high and tends to grow rapidly, especially in such areas as food production, medicine, pharmaceutical and microbiological industries, cosmetology, production of care products and cultivation of plant and animal cells [2]. The main plant-based gelling agents that have received the maximum distribution in Russia include agar-agar and pectin.

Historically, Russia's needs for plant-based gelling agents were covered by domestic producers by no more than 5%. According to estimates of 2023, Russia is among the top 5 leading countries in the consumption of agar-agar and annually acquires 1-1.3 thousand tons of product. The main suppliers are China, Chile, Morocco, Italy and Spain. For 2023, there are two agar-agar plants left in the Russian Federation: the Arkhangelsk Algae Plant, which produces no more than 10 tons of agar, consumed mainly for the production of local marmalade from northern berries. The second is the Korsakov agar plant on Sakhalin, which after three years of repairing communications in 2021 produced the first trial 120-kg batch and has not yet reached the expected design capacity of 30-120 tons per year). The main reason for this condition is the lack of raw materials and labor force.

Even before the collapse of the USSR, the production of pectin at the enterprises of Russia, Moldova, Ukraine was up to 350-400 tons per year (0.20-0.22% of the need); and 1.5-2.0 thousand tons were purchased annually from abroad. Today, the pectin market in Russia is estimated at 12-15 thousand tons per year, which in monetary terms is about 150-180 million US dollars. Over the past 20 years, at least 15 fairly well-known large players in the food market and a number of investors from other industries have tried to organize the production of pectin in Russia, but the projects have not yielded significant results. The main factors hindering the production of domestic pectin are the lack of raw materials: citrus pomace (which is understandable), low cooperation, logistical problems and instability when working with apple pomace and the lack of existing developments in mass production of the technology for obtaining pectin from beet and pumpkin pomace and green pea walls .

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QUALITY EVALUATION OF *ARTEMISIA SEROTINA* BUNGE OIL EXTRACT

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Introduction. At the Department of Engineering disciplines and good practices of the School of Pharmacy of the Kazakh National Medical University named after S.D. Asfendiyarov, full-scale studies of the potential plant material of *Artemisia serotina* Bunge are being carried out [1, 2]. An optimal technology for obtaining an oil extract from the studied raw materials has been developed. The legislative framework of the Republic of Kazakhstan contains a number of regulatory documents regulating the quality of plant extracts (pharmaceutical substances).

The aim is to study and establish the quality parameters of the oil extract from *Artemisia serotina* Bunge.

Materials and methods. The object of the study is an oil extract from *Artemisia serotina* Bunge obtained at the pharmaceutical company FitOleum LLP base (Issyk, Almaty region, Kazakhstan, GMP license No. 18). The reagents used in the analysis were standard, meeting the requirements of the SP RK, the equipment was verified and qualified.

Results. According to our analyzes, the quality indicators of the finished product were determined: description, identification of biologically active substances, density, refractive index, vial volume, acid value, iodine value, microbiological purity, assay, packaging. Long-term stability studies of the finished product are currently underway.

Conclusions. A set of measures was carried out to assess the quality of the *Artemisia serotina* Bunge oil extract: quality indicators were determined and regulated standards were established for them.

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PHYTOCHEMICAL STUDY OF SPRUCE CONES AND EXTRACT BASED ON IT

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Keywords: *pine cones, plant extract, phytochemical study.*

Introduction. The common spruce is one of the main logging breeds on the territory of the European part of the Russian Federation. From one cubic meter of wood, about 250 kilograms of waste wood greens and cones are obtained. As a result of research conducted at the Department of Pharmacognosy, it was revealed that the cones of spruce and the extract obtained from them have pronounced pharmacological activity. It was found that the aqueous extract has a pronounced antioxidant activity, where procyanidins were one of the main groups of BAS [2].

Aim of study. The purpose of our study was to determine the content of BAS in cones in different periods.

Materials and methods. The object of the study are spruce cones harvested on the territory of the Ilyinsky district of Perm Krai from July to March. The extract was obtained by triple extraction with water and water-alcohol mixtures. The determination of the procyanidin content in the cones of the common spruce was carried out using the acid cleavage of procyanidins to anthocyanidins by the Porter method [1]. To determine the antioxidant activity, a reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used.

Results and discussion. According to the results of the analysis of tannins, polysaccharides, procyanidins and other components that affect the pharmacological properties of spruce cones, the data show the need to extend the terms of raw material procurement. The maximum accumulation of procyanidins in the cones of the common spruce is observed in February ($28.1 \pm 1.0\%$). After assessing the content of BAS, it was of interest to determine the antioxidant activity in the extract. It was found that the antioxidant activity of the extract ($IC_{50} = 24.02 \pm 0.68$), close to the known antioxidants ($IC_{50} = 12.63 \pm 0.68$) by December ($IC_{50} = 10.6 \pm 2.29$).

Conclusions. The results obtained show the need to extend the harvesting time of the common spruce cones to obtain extracts with the maximum content of active substances and the greatest antioxidant activity.

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OPTIMISATION OF LACCASE PRODUCTION BY THE LE-BIN 2013 STRAIN OF *JUNGHUHNIA NITIDA* IN SUBMERGED LIQUID CULTURE**Shakhova Nataliya V.** (ORCID: 0000-0002-8733-2168, ResearcherID : H-5513-2013)

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Introduction. Currently, the development of strategies aimed at obtaining new products that minimise or eliminate the use and formation of harmful substances is relevant in industrial production. The use of naturally occurring enzymes is considered as an alternative to chemical catalysis, making the biocatalysis process more ecological and efficient. Laccases produced by basidial fungi are regarded as promising enzymes for green chemistry applications as they are key catalytic oxidants that require only molecular oxygen to function. Furthermore, laccase has a rather high stability, broad substrate specificity and the ability to oxidize, in the presence of appropriate redox mediators, compounds that are not its traditional substrates. Due to these properties, this enzyme is used in various fields of biotechnology, in particular in the cosmetics industry as a biocatalyst in the synthesis of hair dye and in the production of personal care products (toothpaste, laundry detergent) [1]. Laccases derived from edible basidiomycetes are used in the food industry as a stabilising agent for juices and wines [2]. The search for basidiomycete strains with a high oxidative potential is carried out in two interrelated directions: obtaining enzymes with new physico-chemical properties as well as the selection of highly effective "enhancers" and promoters of these enzymes from fungal producers. The use of inducers and optimisation of the cultivation medium makes it possible to significantly increase the laccase activity in the strains-producers in order to achieve the maximum yield of the target enzyme at minimum production costs.

Aim of the Study. The work was aimed to study the effect of submerged cultivation conditions and inducers on laccase biosynthesis by the strain LE-BIN 2013 of *Jungbuhnia nitida*.

Materials and Methods. The object of the study was the strain LE-BIN 2013 of *J. nitida* from the Komarov Botanical Institute Basidiomycetes Culture Collection.

In order to enhance the laccase production by *J. nitida* the method of Full Factorial Design (FFD 3³) for three independent variables was adapted. FFD 3³ was performed according to the technique [3] and included the following variable parameters: carbon source (glucose), nitrogen source (peptone), inducer (CuSO₄), and also macro- and microelements that are necessary for fungal growth. Each variable parameter had 3 levels of variation: +1, 0 and -1. Regression coefficients were calculated and assessed according to the Yates algorithm [3]. The optimum values of each variable parameter were determined by graphical (STATISTICA 8.0 software) and computational methods.

Results and discussion. To refine the optimum medium composition, a regression equation describing the FFD 3³ was calculated. The comparison of calculated and experimental data showed that the coefficient of determination (R²) was 0.8846, which shows an 89% agreement between the experiment and the calculated model.

As a result of solving this equation, the optimum cultivation conditions for the strain LE-BIN 2013 of *J. nitida* were determined, which were 0.22:14.8:4.6 for CuSO₄, glucose and peptone, respectively. The optimisation of the nutrient medium provided a 2-fold increase in the laccase activity of the strain studied.

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IDENTIFICATION OF DIOSMIN IN *HYSSOPUS OFFICINALIS* L. BY ¹H NMR SPECTROSCOPY

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Introduction. Assessment of the content of biologically active substances (BAS) in biomass should be based on environmentally safe, express, precision instrumental methods of analysis. The assortment of such methods is very wide, but all of them have one or another disadvantages. NMR spectroscopy of multicomponent extracts of metabolites is free from most of these disadvantages.

Hyssop has been used for its medicinal properties for centuries. It contains a variety of compounds, including flavonoids, tannins, and essential oils, which are believed to have anti-inflammatory, antiseptic, and expectorant properties [1]. It has been used to treat respiratory infections, digestive problems, and skin conditions. In addition to its medicinal properties, hyssop is also used as a culinary herb [2].

Aim of the Study. The task of this work is to identify of diosmin in leaves of *Hyssopus officinalis* L. by ¹H NMR spectroscopy.

Materials and Methods. Crushed leaves of a commercial sample of hyssop were analyzed in this research. The following solvent and standard sample were used: dimethyl sulfoxide-d₆ (≥99.9%, Sigma-Aldrich, CAS number 2206-27-1) and a standard sample of diosmin (CAS number 520-27-4). The samples were analyzed on a spectrometer JEOL JNM ECA-600, Japan.

Results and discussion. Comparing the ¹H NMR spectra of a *Hyssopus officinalis* L. extract and standard sample of diosmin, we can observe the next signals of diosmin which do not overlap with the signals of other BAS: δ 12.93 (s, 1H, OH-5), 9.46 (s, 1H, OH-3'), 7.57 (dd, J = 8.5, 2.2 Hz, 1H, H-6'), 7.44 (d, J = 2.2 Hz, 1H, H-2'), 7.13 (d, J = 8.7 Hz, 1H, H-5'), 6.82 (s, 1H, H-3), 6.76 (d, J = 2.1 Hz, 1H, H-8), 6.46 (d, J = 2.1 Hz, 1H, H-6). The coupling constants of these signals also coincide. This leads to the conclusion that presented signals can be used to identify diosmin in the extract of *Hyssopus officinalis* L. by ¹H NMR spectroscopy.

Conclusion. In this study identification of diosmin in the extract of *Hyssopus officinalis* L. by ¹H NMR spectroscopy was performed.

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**CO-EXTRACTION OF GLYCYRRHIZIC ACID AND TRACE ELEMENTS
FROM LIQUORICE ROOT USING NATURAL DEEP EUTECTIC SOLVENTS****Shikova V.**¹ (ORCID: 0000-0003-3028-4238, ResearcherID: AFO-2873-2022),**Burakova M.**¹ (ORCID: 0000-0002-3880-0359)¹St. Petersburg State Chemical and Pharmaceutical University
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Introduction. The roots of *Glycyrrhiza glabra* L. are described in traditional Chinese medicine and Russian official medicine [1]. The main biologically active substance of licorice is glycyrrhizin (glycyrrhizic acid and its salts). Natural deep eutectic solvents (NADES) are a combination of a hydrogen bond donor (HBD), which is a saccharide, and a hydrogen bond acceptor (HBA) an organic acid [2]. NADES have become popular for extracting active substances from medicinal plants because they are ecologically friendly. Contamination of phytoextracts with microelements is one of the urgent problems. Trace elements can cause side effects and adversely affect the stability and safety of drugs.

Aim of the Study. To study the ability of NADES for co-extraction of microelements from the roots of *G. glabra* and the potential associated health risk.

Materials and methods. The plant raw material "Licorice Roots" JSC "Krasnogorskleksredstva" was used as the object of the study. Choline chloride (Acros Organics), Sucrose, Sorbitol and Citric acid (all Reachim JSC), Lactic acid (Panreac Química SLU) were used for preparing NADES. The glycyrrhizic acid (GA, 97.1% purity) (Sigma-Aldrich RTC). Chromatographic acetonitrile HPLC (T. Backer).

NADES were obtained by heating a mixture of saccharide and acid at a temperature of 70±2°C with stirring on a magnetic stirrer for 60 minutes [2]. NADES extracts were prepared by maceration with stirring at 600 rpm and constant heating to 50°C for 0.5 h. The concentration of GA was analyzed by HPLC with some modifications [3].

Results and discussion. GA dominates in all NADES extracts, while its concentration in extracts varied from 0.145 to 0.495 mg/g. The yield of GA in NADES based on citric acid was equal to the yield of GA in water, and the efficiency of NADES based on lactic acid was statistically higher compared to NADES based on citric acid. Lactic acid-based NADES has a pH (~1.7) that is near to the pKa cost of GA, which may be one of the reasons for the high extraction yield.

The concentration of metals such as Al, Ca, Cu, Fe, Li, Mg, Mn, Na, and K was analyzed in triplicates using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Perkin Elmer Optima 8000. The extraction of all elements (except Li) by all NADES tested was low (less than 6%), which indicates the non-toxicity of NADES. A direct correlation was found between the content of the element in licorice and the same extracts.

Conclusions. The calculated metal contamination index, hazard index and chronic daily consumption indicate that all *G. glabra* root extracts tested by NADES were non-toxic and should not pose a potential health risk either after topical application or ingestion.

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STUDY OF THE LIPOPHILIC FRACTION OF ALFALFA SICKLE HERB (*MEDICAGO FALCATA* L.)

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Keywords: *Medicago falcata* L., alfalfa sickle, herb, lipophilic fraction, fatty acids, gas-liquid chromatography.

Introduction. The role of fatty acids in the human body is extremely large. Unsaturated fatty acids have a pronounced antisclerotic effect, converting cholesterol into folic acids and promoting their excretion from the body. Polyunsaturated fatty acids improve the therapeutic effect in the treatment of cardiovascular diseases, bronchial asthma, diabetes mellitus, have an immunomodulatory effect. Determination of the fatty acid profile will allow to establish the chemical composition of biologically active substances of a previously little-studied plant – alfalfa sickle.

Aim of the study. To establish the qualitative composition and quantitative content of fatty acids of the lipophilic fraction of the herb *Medicago falcata* L.

Materials and Methods. Composition of fatty acids according to GOST 31665-2012; GOST R 31663-2012; gas-liquid chromatography method; Agilent gas chromatograph, 7890A with Agilent Technologies 7683B series autosampler (USA) and flame ionization detector, Agilent J&W GC Select FAME column 100 m, 25 mm *0.25 microns (Netherlands). Extraction of the lipophilic fraction was carried out using a modified Folch method. The peaks were determined using a standard mixture of FAME 37 components in dichloromethane, Bellefonte, USA.

Results and discussion. The content of the lipophilic fraction in alfalfa sickle grass is 5.05 g / 100 g of raw materials. Fatty acids have been identified in the composition of the lipophilic fraction. Qualitative and quantitative analysis of alfalfa sickle fatty acids by gas-liquid chromatography showed the presence of 15 compounds, which contain from 12 to 24 carbon atoms. The fatty acids of the sickle alfalfa herb were divided into saturated (lauric, myristic, palmitic, margaric, stearic, arachidic, lignoceric) and unsaturated (hexadecene, palmitoleic, oleic, linoleic, α -linolenic, gondoic, erucic). In the composition of saturated fatty acids, acids containing 12, 14, 16, 17, 18, 20, 22, 24 carbon atoms. Palmitic acid has the highest content among them (35.41%). Unsaturated fatty acids are represented by both monounsaturated (hexadecene, palmitoleic, oleic, gondoic, erucic) and polyunsaturated (linoleic, α -linolenic) acids. Among monounsaturated acids, oleic (12.12%) prevails, among polyunsaturated linoleic (11.54%) α -linolenic (8.67%) acids prevail. In this connection, alfalfa sickle grass can be a source of these acids, and it can also be considered as a source of omega-3 fatty acids, because it contains a low coefficient of polyunsaturated acids (linoleic/linolenic).

Sources of funding. The authors state that there is no funding for the study.

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THE EFFECT OF THE LIGHT SPECTRAL COMPOSITION ON THE FORMATION OF VEGETATIVE MASS OF *DIGITALIS PURPUREA* L. PLANTS

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Keywords: *plants, digitalis purpurea, light spectral composition, plant biomass.*

Introduction. Plants are an indispensable source for obtaining many practically valuable substances. Meanwhile, the possibilities of obtaining secondary metabolites in sufficient quantities are often limited. This is due to the reduction in the resources of some valuable wild plants, the belonging of many medicinal plants to endemic groups, rare and endangered species. The solution to this problem is the cultivation of *Digitalis purpurea* L. in artificial conditions. This will allow obtaining the necessary raw materials in large quantities and at any time of the year [1].

Aim. The aim of this work is to study the growing conditions of *Digitalis purpurea* L. at which it is possible to obtain the largest amount of the aboveground part (raw materials), using artificial agricultural systems with different spectral composition of light.

Materials and methods. We conducted an experiment on the effect of the spectral composition of light on the morphophysiological parameters of *Digitalis purpurea* L. variety Lisichka. The following light options were used in the experiment.

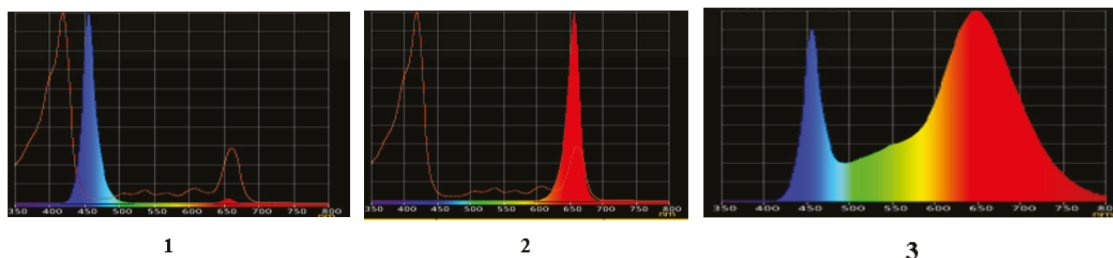


Figure 1. variants of the light under which *Digitalis purpurea* L. plants were grown.
1 – PPFD=60.85 mkmol/m²/s; 2 – PPFD=120.6 mkmol/m²/s; 3 – PPFD=168.4 mkmol/m²/s

Results and discussions.

Table 1 – Morphological indicators of 60 day plants *Digitalis purpurea* L.

Variant	Raw matter of plants, g	Leaf area, sm ²
1 –PPFD=60,85 mkmol/m ² /c	20,34±0,61	401,11±18,2
2–PPFD=120,6 mkmol/m ² /c	13,15±0,33	506±11,4
3 – PPFD=168,4 mkmol/m ² /c	26,41±1,12	618±16,2

The experimental data showed that the effect of the spectral composition of light on the morphological parameters of *Digitalis purpurea* L. throughout the growing season, the plants pf variant PPPFD=168.4 mmol/m²/s was significantly higher than in the plants of variants PPFD=60.85mmol/m²/s PPFD=120.6 mmol/m²/s.

Financing. This study was conducted with the support of the Ministry of Science and High Education of the Russian Federation in accordance with Agreement No. 075-15-2022-317 dated April 20, 2022 and a grant in the form of subsidies from the federal budget of the Russian Federation. The grant was provided for state support for the creation and development of a world-class scientific center: “Agrotechnologies of the future”.

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**BIOLOGICAL ACTIVITY OF *PHLOJODICARPUS* & *AMMI* PLANTS,
AND OBTAINMENT OF THEIR CELL CULTURES**

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Introduction. *Phlojodicarpus sibiricus* and *P. villosus* are Far Eastern endemics, rich in coumarins, widely used in folk medicine as antispasmodic, antioxidant, antibacterial, antitumor agents. *P. sibiricus* was previously included in the Medicines State Register of the Russian Federation. Its rhizomes and roots served to produce the antihypertensive drug Floverin. However, today its production has been suspended due to lack of raw materials, and the species is listed in the Red Books throughout its habitat. The composition of coumarins shown at the *P. sibiricus* and *P. villosus* corresponds to the composition of *Ammi visnaga*. This plant, unlike species under investigation, is successfully grown on plantations. *A. visnaga* cell culture has same biological activity as plant *in vivo*.

Aim of study. To examine antibacterial and antitumor activities of *P. sibiricus*, *P. villosus* extracts, and obtain cell cultures of two *Phlojodicarpus* species and *A. visnaga* with high growth characteristics.

Materials and methods. Objects: *P. sibiricus*, *P. villosus*, *A. visnaga* and their cell cultures. Air-dried roots, leaves and stems of *P. sibiricus*, *P. villosus* were extracted in 96% ethanol at a concentration of 50 mg dry weight per 1 ml of alcohol. All extracts were used for the antibacterial test, and a *P. sibiricus* root extract for MTT testing on the C6 glioma cell line. Callus cultivation was carried out on Murashige-Skoog medium with different combinations of hormones at a temperature of 26 °C and a humidity of 70 ± 5%. The subculture cycle was 28 days for all lines. When reseeded, 1/3 of callus cultures were used. The growth parameters of the line indicated the optimal accumulation of biomass, and the cell viability during the cycle was more than 70%.

Results and discussion. *P. sibiricus* and *P. villosus* extracts obtained from roots, leaves and stems were tested against different bacteria. Big difference in action of the extracts was shown. The best inhibitory effect (more than 50% in comparison with the negative control) was shown by *P. villosus* extracts against *Staphylococcus aureus*, *Salmonella abony*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and only the root extract had a significant effect on *Escherichia coli*. Extracts of *P. sibiricus* showed slight inhibitory effect against all bacteria. According to the results of MTT-test that was performed in 2 repetitions, the *P. sibiricus* air-dried roots extract inhibited C6 glioma cells proliferation by 36%. The different effects of extracts may be due to the composition of coumarins. Callus cultures growth rates characteristics demonstrated that in terms of biomass accumulation, the *A. visnaga* of light cultivation, *P. sibiricus* of root and leaf origin lines are most optimized.

Conclusions. Preliminary tests have shown that *P. villosus* has antibacterial properties against 5 types of bacteria. *P. sibiricus* exhibited cytotoxic properties on C6 glioma cells. As a result of the work, three cell lines *P. sibiricus*, *P. villosus* and *A. visnaga* with high growth characteristics and viability were selected and optimized. In the future, the extracts of these cultures will be used for comparison with previous results on biological activity.

Funding. The study was supported by the Russian Federal Academic Leadership Program Priority 2030.

**ANTIOXIDANT ACTIVITY OF *HERACLEUM SOSNOWSKI* MANDENA COUMARINS
BEFORE AND AFTER ULTRAVIOLET IRRADIATION**

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The article presents the results of determining the antioxidant activity of Sosnowski's hogweed coumarins before and after ultraviolet irradiation.

Keywords: *coumarins, Sosnowski's hogweed, ultraviolet irradiation.*

Introduction. Sosnowsky's hogweed (*Heracleum Sosnowski* Mandena) is a widespread plant in Europe and the CIS. The dominant group of biological active substances is the coumarins, which have specific spectra of pharmacological effects [1].

Aim of study. To determine the antioxidant activity of Sosnowski hogweed coumarins before and after ultraviolet (UV) irradiation.

Materials and methods. For determination of the antioxidant activity, a spectrophotometric method with 2,2-diphenyl-1-picrylhydrazyl reagent is used. The high performance chromatography (HPLC) is used to record the chemical changes that increase with coumarins during UV irradiation.

Results and discussion. It was determined that the antioxidant activity of coumarins before irradiation to UV light is negligible. After irradiation to UV light, the antioxidant activity of umbelliferone and xanthotoxin increased by 2 times, and that of bergapten by 5 times (Figure 1).

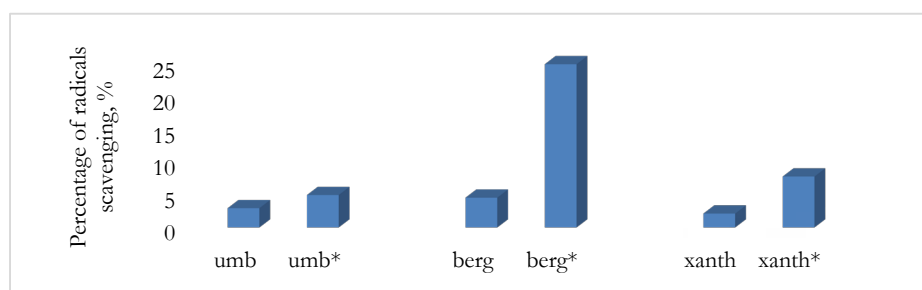


Figure 1. Antioxidant activity of umbelliferone (umb), bergapten (berg) and xanthotoxin (xant) before and after UV irradiation for 6 hours (*)

After HPLC, it was determined that the reason for the increase in antioxidant activity after irradiation to UV light was the formation of new substances (Figure 2). The content of coumarins decreased by 11-47%, which indicates the photodestruction of these substances.

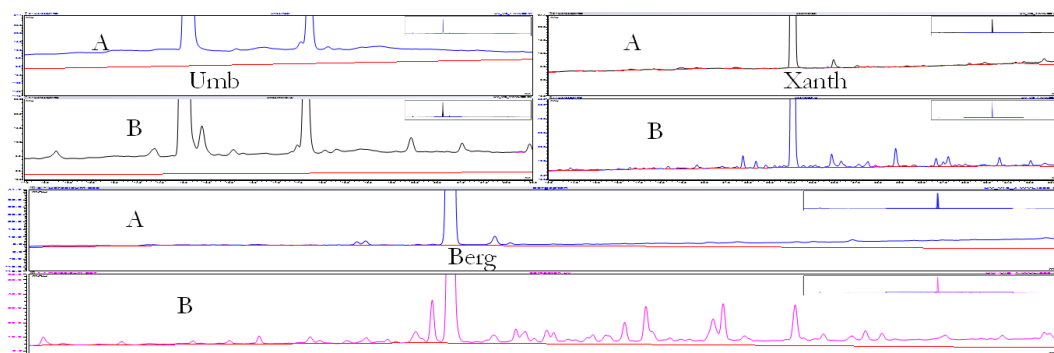


Figure 2. Chromatograms of coumarins before (A) and after (B) UV irradiation

Conclusion. The antioxidant activity of Sosnowski hogweed coumarins is insignificant and significantly increases after irradiation to UV light, which is associated with the destruction of coumarins and the formation of new substances.

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**SECONDARY METABOLITES IN CALLUS CULTURE
OF *SALVIA OFFICINALIS* L. GROWN UNDER LIGHT**
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Introduction. *Salvia officinalis* L. synthesizes a large number of biologically active substances that are successfully used by the pharmaceutical industry for the production of medicines. Leaves of *Salvia officinalis* in Russia are produced from cultivated plants in specialized farms. An alternative source of obtaining biologically active substances (BAS) are callus cultures grown under various conditions, selected to obtain certain groups of secondary metabolites [1].

Aim of the Study. The aim of the study was a qualitative and quantitative analysis of phenolic compounds in the callus culture of *Salvia officinalis*.

Materials and Methods. A callus culture of *Salvia officinalis*, grown under light conditions on a nutrient medium according to the Murashige-Skoog prescription, was used. For the study, ethanol extracts were obtained from dried raw materials. First, qualitative reactions were carried out for the presence of flavonoids – a reaction with a 2% solution of aluminum chloride; 10% sodium hydroxide solution; with a solution of iron ammonium alum; cyanidin test.

Next, a quantitative analysis of flavonoids was carried out by spectrophotometry in terms of rutin. Quantitative determination of the amount of phenolic compounds was carried out by spectrophotometry in terms of chlorogenic acid [2].

Results and discussion. The presence of flavones, flavonols, flavanones, anthocyanins and aurones was revealed in ethanol extracts of light callus culture. When carrying out a quantitative analysis in callus culture, 2.1% of flavonoids in terms of rutin and 1.63% of hydroxycinnamic acids in terms of chlorogenic acid were found.

Conclusions. The presence of phenolic compounds in the dried light callus culture of *Salvia officinalis* was revealed. The presence of biologically active substances was confirmed by the results of a qualitative analysis for the content of flavonoids and hydroxycinnamic acids.

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HPLC ANALYSIS OF CHLOROGENIC ACID IN FOOD CULTURES FOR VARIETAL DEFINITION BENEFITS

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An express high-performance quantitative determination liquid chromatography method of chlorogenic acid was developed and tested on celery leaf. This technique can be recommended for choosing the optimal varieties of food cultures.

Keywords: celery, chlorogenic acid, HPLC, analysis method, varietal benefits, functional food.

Introduction. Chlorogenic acid is a regulator of the gut microflora, which determines a number of its biological effects [1], and can be used as a functional ingredient. Celery accumulates chlorogenic acid and is promising for the development of a functional or specialized food product.

Aim of study. Development of chlorogenic acid HPLC analysis and its approbation on the raw material of celery leaves.

Materials and methods. Dried and crushed raw materials of leaf celery varieties "Zakhar", "Samurai" and "Parus" were exposed to Ultrasound Assisted Extraction with 70% ethanol (1:15) for 20 minutes and then quantitatively diluted 10 times with deionized water. A solution of chlorogenic acid (Sigma Aldrich) 0.00214 mg/ml was used as a reference sample (RS). Quantification was carried out on a Prominence LC-20 chromatograph (Shimadzu, Japan) with a diode array detection (wavelength 270 nm), an Intersil 5 μ m ODS-3 100 \AA 250*4.6 m column. Mobile phase: acetonitrile (A) and trifluoroacetic acid 0.03% (B). Elution mode: 0-5 min. 15% A, 5-12 min. 30% A.

Results and discussion. Chromatograms of chlorogenic acid RS and alcohol extract of "Zakhar" variety celery (test sample) are shown in the figure.

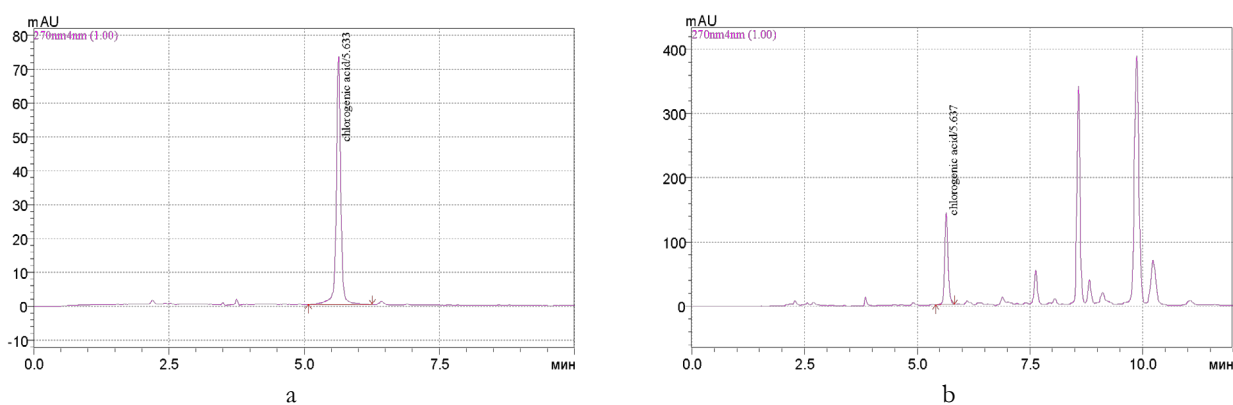


Figure. (a) – chromatogram of the RS, (b) – chromatogram of test sample

The proposed chromatographic conditions have a number of advantages: the use of gentle reagents, fast chromatogram recording time, and high separating power. Approbation of the technique showed that the greatest accumulation of chlorogenic acid is observed in the variety "Zakhar" ($0,75 \pm 0,05\%$), while in the varieties "Parus" and "Samurai" it was $0,31 \pm 0,05\%$ and $0,22 \pm 0,06\%$ respectively.

Conclusion. The method for the quantitative determination of chlorogenic acid is characterized by resource saving and demonstrates the rapid determination of a substance in plant materials. The studied celery varieties accumulate different amounts of the target component, which makes the analysis of varietal advantages relevant. Further transfer of the proposed methodology to other food cultures will allow us to recommend a new approach for assessing the nutritional value of agricultural crops.

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ОПРЕДЕЛЕНИЕ АНТИОКСИДАНТНОГО (ГЕРОПРОТЕКТОРНОГО) ПОТЕНЦИАЛА *ALNUS GLUTINOSA*

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Аннотация. Экстракты *Alnus glutinosa* являются потенциальными соединениями с антиоксидантным и геропротекторным свойствами. Установлено, что по отношению к радикалам ABTS и DPPH и по отношению к FRAP проявляется значительная антиоксидантная активность: $1094,02 \pm 14,53$ мкмоль экв. тролокса/г, $584,45 \pm 35,31$ мкмоль экв. тролокса/г и $471,63 \pm 7,06$ мкмоль экв. тролокса/г, соответственно.

Ключевые слова: *Alnus glutinosa*, антиоксидантная, геропротекторная активность, экстракты.

Введение. Среди ценных источников геропротекторов могут быть лекарственные растения, которые веками использовались в народной медицине [1]. Их использование обусловлено тем, что, поскольку растительные экстракты одновременно воздействуют на сотни мишеней в геноме человека, их свойства позволяют сохранять значительный геропротекторный потенциал. С медицинской точки зрения, частичное ингибирование нескольких мишеней может быть более эффективным, чем полное ингибирование одной мишени, что делает растительные экстракты перспективными агентами с геропротекторным механизмом действия [2]. Часто геропротекторные свойства лекарственных растений связывают с их антиоксидантной активностью. Таким лекарственным растением является *Alnus glutinosa*.

Целью работы является скрининг образцов экстрактов ольхи черной (*Alnus glutinosa*) *in vitro* на предмет их антиоксидантного (геропротекторного) действия.

Материалы и методы. Антиоксидантную активность экстрактов растительных образцов определяли по способности улавливать свободные радикалы DPPH (2,2-дифенил-1-пикрилгидразила) и ABTS (2,2'-азино-бис(3-этилбензотиазолин-6-сульфоновой кислоты), а также по восстановительной активности при взаимодействии с комплексом Fe (III)-2,4,6-трипиридил-*s*-триазин (FRAP). Все спектрофотометрические измерения проводили с использованием микропланшетного ридера CLARIOstar (BMG Labtech, Германия).

Результаты и обсуждение. Результаты определения антиоксидантной активности *Alnus glutinosa* представлены в таблице.

Таблица – Антиоксидантная активность экстрактов *Alnus glutinosa*

№ п/п	Виды антиоксидантной активности	Значение, мкмоль экв. тролокса/г
1	ABTS	579,07±41,87
2	DPPH	275,89±23,55
3	FRAP	378,69±31,03

Из табличных данных следует, что экстракты *Alnus glutinosa* обладают значительной антиоксидантной активностью по отношению к радикалам ABTS и DPPH и восстанавливающей способностью FRAP: $1094,02 \pm 14,53$ мкмоль экв. тролокса/г, $584,45 \pm 35,31$ мкмоль экв. тролокса/г и $471,63 \pm 7,06$ мкмоль экв. тролокса/г, соответственно.

Выводы. Таким образом, получены результаты скрининга экстрактов на наличие и степень выраженности антиоксидантных (геропротекторных) свойств *in vitro*.

Финансирование. Работа выполнена при финансовой поддержке Российского научного фонда (соглашение №21-76-10055).

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AMELIORATIVE EFFECTS OF VITAMINS-LOADED FLAVOURED NANOPHYTOSOMES FORTIFIED WITH ZINC AND STAR ANISE VOLATILE OIL AGAINST CSA-INDUCED LIVER AND KIDNEY INJURY IN RATS: APPLICATION IN FUNCTIONAL ICE CREAM

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Keywords: *Flavoured nanophytosome, vitamins, Immune system, Star anise, Ice cream, phytosome encapsulation.*

Abstract. The present study aims to investigate the potential protective and ameliorative effects of phytosomes on the encapsulation of vitamins (A, E, D, B complex, Folic acid, and C) and their mixture with zinc as an antioxidant and an anti-inflammatory agent against CsA-induced liver and kidney injury. The phytosomes were flavoured with star anise volatile oil to mask the vitamins' flavour and unacceptable taste. The star anise volatile oil was added as a natural flavour to ice cream, which served as a food model fortified with vitamins-loaded flavoured nanophytosomes (VFnpPs) at two different concentrations (0.166g and 0.330g). The VFnpPs were characterized in terms of particle size, zeta potential, encapsulation efficiency (EE), and transmission electron microscope (TEM). Star anise oil compounds were identified and quantified using GC-MS. Male rats were randomly divided into three groups: control normal group received orally 1 ml of saline, the second group, immunosuppressive group received cyclosporine-A (CsA) orally in a dose of 15 mg/kg body weight daily for 8 weeks, and the third group received CsA simultaneously with the VFnpPs (3 mg of the VFnpPs) daily for 8 weeks. Treatment of rats with CsA alone resulted in a significant increase in the levels of creatinine, urea, and malondialdehyde (MDA), as well as the activities of aspartate transaminase (AST) and alanine transaminase (ALT). Meanwhile, the level of superoxide dismutase (SOD), catalase (CAT), glutathione S. transferase (GST), proteins, CD4, INF- γ , IL-6, IL-1 β , and TLR4 decreased. However, the group that received CsA simultaneously with VFnpPs showed a significant decrease in the levels of creatinine, urea, and MDA, as well as the activities of AST and ALT. Moreover, the levels of SOD, CAT, GST, proteins, CD4, INF- γ , IL-6, IL-1 β , and TLR4 were significantly increased. The increase in the ratio of VFnpPs had little effect on the physicochemical and sensory evaluation of the ice cream. Overall, the study suggests that VFnpPs could potentially protect against CsA-induced liver and kidney injury and serve as a promising natural therapy for treating such conditions.

**NEUROPROTECTIVE AND ANTIHERPETIC PROPERTIES OF POLYPHENOLIC COMPOUNDS
FROM MAACKIA AMURENSIS HEARTWOOD**

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Introduction. *Maackia amurensis* Rupr et Maxim. is an endemic woody plant of the Fabaceae family, widespread in the Russian Far East. The polyphenolic complex of *M. amurensis* heartwood is used to produce drug Maksar[®]. The active compounds of this complex are isoflavones, pterocarpan, flavanones, isoflavans, isoflavanones, chalcones, lignans, and monomeric and dimeric stilbenes.

Aim of the Study. The aim of this study was to assess the antiherpetic activity of polyphenolic compounds, the constituents of Maksar[®], as well as their ability to reduce the level of intracellular reactive oxygen species (ROS) and increase mitochondrial membrane potential.

Materials and Methods. The structures of polyphenolic compounds from *M. amurensis* were elucidated using NMR, mass-spectrometry circular dichroism (CD) techniques. We used DPPH (2,2-diphenyl-1-picrylhydrazyl) and ferric reducing power (FRAP) tests to evaluate antioxidant activity of polyphenolic compounds. The antiviral activity of stilbenes from *M. amurensis* against herpes simplex virus type 1 (HSV-1) was assessed using the cytopathic effect inhibition and real-time PCR assays. A model of paraquat (PQ)-induced neurotoxicity was used to study the neuroprotective potential of polyphenolic compounds from *M. amurensis*.

Results and discussion. In this study, we isolated a new isoflavanostilbene maackiapicevestitol (**1**) (Fig.) as a mixture of two stable conformers **1a** and **1b** as well as five previously known dimeric and monomeric stilbens: piceatannol (**2**), maackin (**3**), scirpusin A (**4**), maackiasine (**5**), and maackolin (**6**) from *M. amurensis* heartwood. The absolute configuration of asymmetric centers in compound **1** was determined as 3*R*,4*S*. Maksar[®] and polyphenolics **1–6** isolated from *M. amurensis* heartwood possessed moderate anti-HSV-1 activity in cytopathic effect (CPE) inhibition and PCR assays. Stilbenolignan maackolin (**6**) at a concentration of 10 μM, also effectively increased the viability of PQ-treated Neuro-2a cells (by 16%) at a concentration up to 10 μM, which may be due to the ability of this compound to increase the value of mitochondrial membrane potential. Although compounds **1–6** possessed high DPPH-scavenging effect and FRAP values, only compounds **1** and **4** at a concentration of 10 μM as well as Maksar[®] (10 μg/mL) statistically significantly reduced the level of intracellular ROS in PQ-treated Neuro-2a cells. Dimeric stilbene scirpusin A (**4**) effectively increased mitochondrial membrane potential.

Thus, Maksar[®] and its components possessed significant neuroprotective potential and moderate antiherpetic properties. These results open perspectives to investigate the potential of Maksar[®] for new medical applications.

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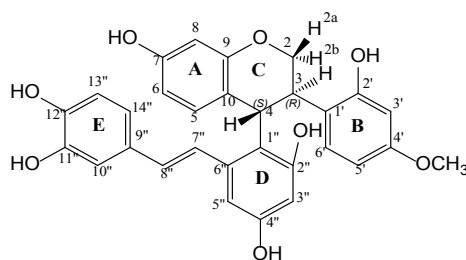


Figure. Chemical structure of compound 1

**IN VITRO AND IN SILICO BIOLOGICAL ACTIVITY OF EPOXY-LIGNAN
AND OTHER COMPOUNDS FROM *CADIA PURPUREA*****Tsegu Kiros¹, Rajalakshmanan Eswaramoorthy², Seid Mohammed³, Aman Dekebo⁴, Yadessa Melaku⁴**¹Central Laboratory and Chemistry Department, Haramaya University, Dire Dawa, P.O.Box.138, Ethiopia;²Department of Biomaterials, Saveetha Dental College and Hospitals,
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Introduction. *Cadia purpurea* (Fabaceae) is a poisonous green shrub distributed in Ethiopia, Eritrea, Yemen, Oman, North Somalia and North Kenya [1]. In Ethiopia, people use roots of *C. purpurea* to cure severe wounds [2] and the powder leaves along with coffee to heal fire burned body [3]. Despite the extensive use of this plant as a remedy against various ailments in Ethiopia, reports on phytochemical constituents and biological activities of the leaves and roots parts were minimal.

Aim of the study. This study focused on the investigation of chemical constituents and evaluation of their *in vitro* and *in silico* antibacterial and antioxidant activities from roots and leaves of *Cadia purpurea*.

Materials and methods. The structures of isolated compounds were established based on the FT-IR, GC-MS and NMR instrumental analysis and comparison with reported data. The *in vitro* antibacterial and antioxidant activities of extracts and isolated compounds were evaluated against standard human bacterial pathogens. The *in silico* molecular modeling of the isolated compounds was studied against *E. coli* gyrase B (PDB ID: 6F86), *S. aureus* pyruvate kinase (PDB ID: 3T07), *P. aeruginosa* PqsA (5OE3) and human peroxiredoxin 5 (PDB ID: 1HD2) protein models.

Results and discussion. The present findings disclosed that the isolated compounds **1-5** and extracts showed dose-dependent antibacterial activity with a better antibacterial activity displayed by calpurnine (**3**) and apigenin-7-*O*-neohesperidoside (**2**) against *E. coli* (18.5 ± 0.02 mm and 12.1 ± 0.1 mm, respectively) at a maximum concentration (1 mg/mL). All extracts and isolated compounds displayed better antibacterial activity against *P. aeruginosa* strain than chloramphenicol (7.2 ± 0.6 to 8.2 ± 0.6 mm) almost at all tested concentrations. The molecular docking result revealed that compound **1**, calpurnine (**3**) and compound **4** showed stronger binding affinity to *E. coli* gyrase B (6F86) protein model than chloramphenicol. Whereas all the compounds **1-5** showed a better binding energy to *S. aureus* pyruvate kinase (3T07) and human peroxiredoxin 5 (1HD2) than chloramphenicol and ascorbic acid, respectively. Compounds **1-4** also recorded higher docking score against *P. aeruginosa* PqsA (5OE3) than chloramphenicol.

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CYTOTOXIC ACTIVITY *IN VITRO* OF *LAMIUM MACULATUM* EXTRACT

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Introduction. Aqueous extracts of *L-album* cause the death of B16 mouse melanoma cells, methanol and chloroform extracts penetrate lung tumor cells, but do not inhibit normal cells. The ethyl acetate extract of *L.album* is toxic to fibroblasts [1]. On the Russian pharmaceutical market there are fees «For polycystosis», «For leukemia», which have *L.album* in the composition. So, there is information about the cytotoxic activity of the *Lamium* type, but the cytotoxic activity of *L. maculatum* is not described in the literature.

Aim of Study. To study the dose dependence of the cytotoxic effect of the extract of *L.maculatum* in comparison with doxorubicin.

Materials and Methods. A sample of raw material weighing 0.2 g was extracted with 70% ethanol in a water bath for 30 minutes at 60°C and the ratio of raw material:extractant 1:50. Cells were seeded into wells of a 96-well plate for adhesion. A day later, the medium was changed in each variant of the experiment and the extracts were added. Incubated for 72 hours in a CO₂ incubator (5%, +37°C). The medium was removed from all wells. A 10% solution of 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, 5 mg/ml was added to each well. on phenol red-free DMEM culture medium. After 4 h of incubation at 5% CO₂ and +37°C, the medium was taken and 100 µl of DMSO (dimethyl sulfoxide) solution was added to each well. Incubated for 10 min on a shaker at room temperature. The optical density of the resulting formazan solution in DMSO was measured on a SUNRISE plate spectrophotometer at a wavelength of 492 nm.

Results and discussion. There is an increase in the inhibition of cell growth with an increase of concentration from 1,25 µM to 7,5 µM in cell cultures Hela (from 5,0% to 85,4%), MeWo (from 1,8% to 56,9%), Bj-hTERT (from 1,3% to 68,4%).

A relationship was found between the increase in concentration and the percentage of growth inhibition cell line Hela ($F = 1.784 < F_{cr} = 5.987$), MeWo ($F = 0.299 < F_{cr} = 5.987$), Bj-hTERT ($F = 0.540 < F_{cr} = 5.987$).

On the MeWo cell line, the cytotoxic activity of doxorubicin (79,8-81,7%) exceeds the activity of *L. maculatum* extract (1,8-56,9%) in the entire range of concentrations.

On Bj-hTERT cell line, the cytotoxic activity of the extract exceeds the activity of doxorubicin starting at a concentration of 5 µM, reaching 2.4 times (29,0% for 10 µM doxorubicin and 68,4% for 7.5 µM *L. maculatum* extract).

On Hela cell lines, the cytotoxic activity of the extract exceeds the activity of doxorubicin starting from a concentration of 5 µM, reaching 1.4 times (62,9% for 10 µM doxorubicin and 5,4% for 7.5 µM *L. maculatum* extract).

Conclusions. The dose-dependent cytotoxic activity of a 40% water-ethanol extract of *Lamium maculatum*. was demonstrated on cell lines Hela, MeWo, Bj-hTERT. On cell lines Hela, Bj-hTERT the cytotoxic activity exceeded the activity of doxorubicin in concentrations of 5 and 7.5 µM.

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DETERMINATION OF THE PHENOLIC COMPOUNDS CONTENT AND ANTIOXIDANT ACTIVITY OF
AURICULARIA POLYTRICHA GROWN ON ASPEN

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Keywords: *antioxidant activity, thick-haired auricularia, fruit bodies, extraction, water and water-alcohol extracts, phenolic compounds.*

Introduction. *Auricularia polytricha* is a basidial mushroom that grows on tree trunks and branches and is found in broad-leaved forests of tropical regions of the world. In Belarus, it is cultivated on artificial nutrient media as a food mushroom [1]. In addition to nutritional value, auricularia has a number of pharmacological actions. In this regard, it is important to study this mushroom as a source of biologically active substances with antioxidant activity [2].

Aim of study. Determination of the phenolic compounds content and antioxidant activity of aqueous and water-alcohol extracts obtained from *Auricularia polytricha* grown on aspen and aspen sawdust.

Materials and Methods. Dried fruit bodies of *Auricularia polytricha* of two strains 174 and 175; extractants: purified water, ethyl alcohol 40%, 70% and 96%. The raw materials were pre-ground to powder. Aqueous and water-alcohol extracts were prepared at a ratio of raw materials and extractant 1:50, they were used for qualitative and quantitative analysis of phenolic compounds and determination of antioxidant activity [3].

Results and discussion. In extracts obtained by extracting 96% alcohol from *Auricularia polytricha* grown in vivo, presumably luteolin-7-glucoside was found. Substances with maximum absorption in the region of 550 nm were found in all water-alcohol extracts of auricularia.

The content of phenolic compounds in extracts of 174 strains of auricularia grown in vivo ranged from 3.67 mg% to 6.41 mg%, and in 175 strains from 3.69 mg% to 4.88 mg%; aspen grown on sawdust: from strain 174 ranged from 9.75 mg% to 14.09 mg%; strain 175 – from 8.70 mg% to 14.55 mg%.

The antioxidant activity of extracts obtained from auricularia grown in vivo from strain 174 ranged from 4.5% to 19.8%, strain 175 – from 7.3% to 16.1%; aspen grown on sawdust: from strain 174 ranged from 17.1% to 49.6%; strain 175 – from 15.6% to 46.4%.

Conclusions. Antioxidant activity, qualitative and quantitative composition of *Auricularia polytricha* fruit bodies grown in natural conditions and on aspen sawdust were determined.

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PHYTOCHEMICAL ANALYSIS OF *CIRSIUM* SPECIES

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Keywords: *Cirsium*, flavonoids, hydroxycinnamic acids.

Introduction. Plants of the genus *Cirsium* are widespread everywhere. Flavonoids (apigenin, luteolin, rutin, hyperoside, quercetin, etc.) and hydroxycinnamic acids (chlorogenic, caffeic, gallic acids, etc.) were found in their composition [1]. Plants of *Cirsium* species exhibit antioxidant, antimicrobial and anti-inflammatory activity and have a wide resource potential for harvesting plant raw materials [2].

The aim of the study is to determine which *Cirsium* species contain the largest amount of flavonoids and hydroxycinnamic acids and which *Cirsium* species has the most diverse chemical composition.

Materials and methods. The object of the study is the herb of *C. palustre*, *C. canum*, *C. oleraceum*, *C. vulgare* and *C. arvense*. The plant raw materials were harvested in places of natural growth in the vicinity of Minsk in the summer during the flowering period and subjected to air-shade drying. To obtain water-alcohol extracts plant raw materials weighing 0.400 g was extracted with ethanol in a volume fraction of 40%, 70% and 96% at a ratio of plant raw materials and extractant of 1 to 25 for 1 hour in a water bath at a temperature of 65 °C. The amount of flavonoids and hydroxycinnamic acids was measured by spectrophotometry. To measure the amount of flavonoids 96% ethanol, diluted acetic acid, a solution of 50 g/l aluminum chloride in 70% ethanol and a solution of 50 g/l hexamethylenetetramine were used. To measure the amount of hydroxycinnamic acids 0.5 M hydrochloric acid solution, Arnov reagent and diluted sodium hydroxide solution were used. High-performance liquid chromatography (HPLC) was performed to study the composition of flavonoids.

Results and discussion. The best flavonoid extractants are 40% ethanol for extracts of *C. palustre* (0.71%) and *C. oleraceum* (1.47%) and 70% ethanol for extracts of *C. canum* (0.67%). The amount of flavonoids in 40% and 70% ethanol extracts of *C. vulgare* and *C. arvense* is the same (*C. vulgare* – 0.37%, *C. arvense* – 0.56%). The largest amount of flavonoids in terms of rutin is contained in the herb of *C. oleraceum* (1.47%). 40% ethanol is the best extractant for hydroxycinnamic acids of *C. palustre* (0.34%), *C. oleraceum* (0.80%) and *C. arvense* (0.73%), 70% ethanol is the best extractant for hydroxycinnamic acids of *C. canum* (0.54%) and *C. vulgare* (0.50%). The largest amount of hydroxycinnamic acids in terms of chlorogenic acid is contained in the herb of *C. oleraceum* (0.80%). Rutin and luteolin-7-glucoside were detected by HPLC in the herb of all analyzed *Cirsium* species. Quercetin, kaempferol and its derivative were detected in the herb of *C. palustre*. Nicotiflorin was identified in the herb of *C. oleraceum*.

Conclusions. The largest amount of flavonoids (1.47% in terms of rutin) and hydroxycinnamic acids (0.80% in terms of chlorogenic acid) is contained in the herb of *C. oleraceum*. The largest number of flavonoids was identified in the herb of *C. palustre* (5 flavonoids).

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PLANTS FROM THE REGIONS OF INNER ASIA AND THEIR BIOSYNTHETIC POTENTIAL

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Introduction. According to WHO statistics, the leading causes of death worldwide remain such groups of socially significant diseases as cardiovascular, infectious, neurodegenerative and oncological. However, since 2020, the coronavirus (COVID-19) outbreak has been recognized as an international health emergency. Therefore, research aimed at developing innovative drugs with antibacterial, antitumor, neuroprotective, anti-inflammatory, and other activities remains relevant all over the world.

It is known that natural sources are the best resource for the search for new agents, which, in collaboration with synthetic chemists and biologist, determine the potential for the discovery of new structures, which can subsequently lead to the production of effective drugs for the treatment of various human diseases. Thus, in recent years, more and more attention of scientists around the world has been directed to the study of the experience of Oriental medicine, based on the use of natural biologically active substances. Integration of the experience of Oriental medicine with the achievements of modern science contributes to the search for a leading compound as the basis of an innovative drug.

Aim of study. The main aim of the research is to search for and create leading compounds from among natural and semi-natural compounds as a scientific basis for the development of national innovative drugs for treatment of socially significant diseases.

Materials and methods. The objects of the study are the Umbelliferae, Rutaceae, Fabaceae, Ranunculaceae and Asteraceae families' plants from the regions of Inner Asia as a source of compounds of terpene, phenolic nature as well as alkaloids.

Results and discussion. Qualitative reactions proved the presence of saponins, essential oils, tannins, flavonoids, coumarins, polysaccharides in the studied species. The MALDI_TOF-MS method revealed saikosaponins in the roots of *B. bicaule*, the highest content of which was determined in cork. The composition of essential oils *A. scoparia*, *B. bicaule* (in different phenophases), *K. baicalensis* from Buryatian flora was studied. The antibacterial activity against gram-positive, gram-negative bacteria and fungi of *K. baicalensis* essential oils was determined. The composition of the lipophilic fraction of *O. lanata*, *O. caespitosa*, *O. myriophylla*, *O. squamulosa*, *A. anethifolia*, *A. desertorum*, *A. pubescens*, *A. scoparia* (in different phenophases), *R. circinatus* и *C. dauricum* was determined for the first time. It has been shown that the main components were PUFAs, also plants of the genus *Oxytropis* L. contain a high amount of benzoic acid. The content of rutin and chlorogenic acid in plants of the genus *Artemisia* L. was determined, the highest content was noted in *A. dracunculus*. Scoparone was isolated from the aerial part of *A. scoparia*. From the roots of *H. dauricum* difilin, haplomyrtine, isodaurinol, skimmianin were isolated, which have cytotoxic activity against breast cancer cells. A new method of tincture preparation in the base of *S. divaricata* roots and *A. frigida* herb were developed. The results of the primary introduction of *A. jacutica* as a source of chamazulene with anti-inflammatory activity indicate the prospects of introducing this species into the culture in the natural and climatic conditions Buryatia.

Conclusions. The results obtained have practical importance in the field of creating leading compounds based on natural biologically active substances and developing innovative medicine remedies.

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THE OPTIMIZED PROTOCOL FOR GINSENOSE TYPE AND GROUPS QUANTIFICATION IN BIOREACTOR-CULTURED CELLS OF *PANAX JAPONICUS* BY UPLC-ESI-MS**Tyurina Tatiana^{1,2}, Klychnikov Oleg^{1,2}, Metalnikov Pavel¹, Titova Maria¹**¹M.V. Lomonosov Moscow State University
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Introduction. *Panax japonicus* (T. Nees) C.A. Mey. is a medicinal ginseng species rich in ginsenosides of different types. Cell culture of *P. japonicus* grown in large-scale bioreactors is a promising alternative to wild and plantation-cultivated plants as a ginsenosides source. Because of its high biomass productivity and diverse ginsenoside profile, *P. japonicus* cell biomass is a potentially valuable component of functional foods, food additives, and natural health products. However, cell cultures are not a complete analog of wild plants and can yield different spectra of ginsenosides in a high range of concentrations, and these substances can have varying biological activity. To summarize, (bio)chemical identification of different ginsenoside compounds, including isomers, in bioreactor-grown cells is required before it can be authorized for use in pharmacological and health products.

Aim of the Study. This study aims to evaluate the no-loss sample preparation protocol for different types and groups of ginsenosides isolated from *P. japonicus* suspension cell cultures grown in bioreactors and their quantitative UPLC-MS identification.

Materials and Methods. Suspension cell culture of *P. japonicus* was grown sequentially in a series of laboratory (20-L), pilot (75-L), and industrial (630-L) bioreactors. Freeze-dried powdered cell biomass was extracted with 70% (v/v) aqueous methanol in an ultrasonic bath; the extracts were centrifuged and evaporated. Dried samples were resuspended in 5% (v/v) aqueous acetic acid solution, SPE purified on a C-18 cartridge (Supelco, USA), evaporated, and stored at -18 °C. Before the analysis, samples were dissolved in 70% (v/v) aqueous methanol with 0.1% formic acid. Phytochemical analysis of cell biomass for secondary metabolites was performed using ultra-performance liquid chromatography–electrospray ionization–mass spectrometry (UPLC–ESI–MS). Quantitative analysis was performed using a mixture of ginsenosides as an external standard. Calculation of peak height and area was performed using MassLynx software (Waters, USA).

Results and Discussion. Suspension cells cultured in bioreactors showed intensive growth. Cell biomass accumulated a broad spectrum of ginsenosides, including the major ginsenosides (Rb1, Rb2, Rb3, Rc, Rd, Rg3, Re, Rf, Rg1, Rh1, R0) at a total concentration of 52.4 ± 4.5 mg/g DW and their isomers and derivatives at sum concentration of 47.1 ± 4.4 mg/g DW.

Some of these molecules exist as isomers, e.g., Rf and PseudoF11, with the same molecular mass unresolved in MS¹ and similar elution time at conventional HPLC separation protocols. We optimized the protocol of LC separation to achieve baseline separation of these isomers. Due to the high concentration dynamic range of our target molecules (more than four orders of magnitude), we had to optimize the sample amount to quantitatively approach ginsenosides presented in samples at low and high concentrations in one LC-MS separation. Using high mass resolution and non-destructive chemical ionization methods, we could detect 30 different ginsenoside compounds in *P. japonicus* cultured cells. We could estimate the absolute quantity of these molecules in our cell samples using external and internal calibration based on standard ginsenosides of different origins. In conclusion, the optimized method allows identification and quantification of different groups of ginsenosides and their isomers in bioreactor-grown cell biomass of *P. japonicus* and can be used for quality control in bioreactor production systems.

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**APPLICATION OF NMR SPECTROSCOPY FOR DETERMINATION
OF BIOLOGICALLY ACTIVE SUBSTANCES IN OBJECTS OF PLANT ORIGIN**

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Introduction. Different physicochemical methods, such as HPLC and spectrophotometry, are used to determine various biologically active substances of plant origin. These methods require complex sample preparation and the availability of rare and expensive authentic standard samples that are not available for many biologically active substances. In this research, an alternative NMR spectroscopy method for determination of anthraquinone derivatives in roots and rhizomes of *Rubia tinctorum* L., which does not require difficult and time-consuming sample preparation and, most importantly, authentic standard samples, is proposed.

Rubia tinctorum L. is a perennial herbaceous plant of the Rubia family, up to 2 m high [1]. It grows in steppes and in forests, riverine shrub thickets, on the sides of irrigation canals. *Rubia tinctorum* L. rhizomes and roots contain about 5-6 % anthraquinone derivatives, flavonoids, iridoids, organic acids [2]. Rubia species produce a number of phytochemicals, including anthraquinone derivatives, which are of interest to pharmaceutical researchers.

Aim of the Study. The task of this work is to develop an alternative ¹H NMR spectroscopy method for identification and quantitative determination of ruberythric acid and lucidin-3-primveroside in roots and rhizomes of *Rubia tinctorum* L.

Materials and Methods. Crushed roots and rhizomes of *Rubia tinctorum* L. were analyzed in this research. Dimethyl sulfoxide-d₆ (≥99.9%, Sigma-Aldrich, CAS number 2206-27-1) was used as a solvent. And also standard samples of ruberythric acid (CAS № 152-84-1) and lucidin-3-primveroside (CAS № 29706-59-0) are used. The samples were analyzed using a JEOL JNM ECA-600 NMR spectrometer (Japan).

Results and discussion. Comparing the ¹H NMR spectra of a *Rubia tinctorum* L. extract and standard sample of ruberythric acid, the next signals can be observed: two doublet proton signals at 7.62 ppm and 7.74 ppm (J = 8.5 Hz each) in the ¹H NMR spectrum of the standard sample of ruberythric acid and 7.62 ppm (J = 8.5 Hz) in the ¹H NMR spectrum of the *Rubia tinctorum* L. extract, which do not overlap with other aromatic proton signals. The coupling constants of these doublet signals also coincide. A non-overlapping singlet signal at 7.48 ppm in the ¹H NMR spectra of lucidin-3-primveroside and the *Rubia tinctorum* L. extract are also observed. This suggests that the doublet signal at 7.62 ppm and the singlet signal at 7.48 ppm can be used to identify and quantify ruberythric acid and lucidin-3-primveroside, respectively, in the *Rubia tinctorum* L. extract by ¹H NMR spectroscopy.

Conclusion. In this study an alternative ¹H NMR spectroscopy method for identification and quantitative determination of ruberythric acid and lucidin-3-primveroside in roots and rhizomes of *Rubia tinctorum* L. was developed.

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BACTERICIDAL AND FUNGICIDAL PROPERTIES OF EXTRACT TAKEN FROM NONEA ROSSICA HERB

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Introduction. One of the fundamental tasks of modern medicine is the search for new drugs with antibacterial and antifungal effects. According to traditional medicine, plants of the genus *Nonea* have anti-inflammatory and antimicrobial properties, therefore they are used in the treatment of infected wounds. Taking into note the experience of use similar medicines in folk medicine and phylogenetic relationship studied plant with other species of the genus, research of *Nonea rossica* Steven is significant interest for screening the pharmacological properties nonea, on the basis of which effective medicines can be created.

Aim of the Study. In this work, we research a pharmacological activity phytomedicines taken from herb raw material of *Nonea rossica* against pathogenic microbes and fungi.

Materials and Methods. Samples of the studied plants were collected in the Novosibirsk region (N 55°31', E 82°57') on a typical habitat (steppe meadow) during the flowering period in 2022. The collected raw materials were brought to an air-dry state and crushed. Extracts were obtained from raw material samples using ethyl alcohol of various concentrations: 70%, 40% and 20%. To obtain extracts, accurate weights of crushed raw materials were placed in a flask and filled with extractant in a ratio of 1:10. The flasks with attached reflux condensers were kept in a water bath at a temperature of 55 °C for 60 minutes, and after cooling they were filtered. Antimicrobial activity was determined by the serial dilution method [1]. At the same time, strains of microorganisms were cultivated on nutrient media, to which the studied extract was added. Strains of gram-positive bacteria (*Staphylococcus aureus* ATCC 6538 FDA 209P and *Bacillus cereus* ATCC 10702), as well as fungi of the genus *Candida albicans* NCTC 885-653. The cultivation of microorganisms was carried out on Muller-Hinton agar and broth media under aerobic conditions at a temperature of +37°C. The cultivation time was 1–2 days. The analysis of antibacterial activity was carried out by the method of serial dilutions in a liquid medium in a total volume of 1 ml.

Results and discussion. As a result it was established the extracts taken with using 40% and 70% ethyl alcagol completely inhibited the growth of cultures: *S.aureus*, *B.cereus* and *C.albicans*, while the extract obtained using 20% ethanol as an extractant, don't showed inhibitory properties.

Previously, our studies have established that *N.rossica* contains dehydropyrrolizidine alkaloids, caffeic acid esters, rosmarinic acid and its derivatives, as well as O-glycosides of quercetin and kaempferol, and a new compound, noneazid, which is a quercetin derivative acylated with a fragment of caffeic acid [2].

It seems highly probable ehe established bacteriocidic activity in relation to *S.aureus*, *B.cereus* and fungicide activity in relation to *C.albicans* may be associated with the presence in the composition of the phenolic complex – noneazid and caffeic acid, as well as substances having in their structure lacton ringes (similar coumarin stucture). It should be noted that noneazid also contains caffeyl, a radical of caffeic acid, which, upon acid hydrolysis of noneazid in the gastrointestinal tract, will be cleaved from its structure with the formation of caffeic acid and thereby enhance antibacterial activity..

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SCREENING FOR ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF FUNGI

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Introduction. At present, more than 130 pharmacological effects have been identified in fungi, including antifungal and antioxidant activity [1]. Since they are expressed to different degrees in different fungi, it is necessary to conduct research and identify representatives with the greatest pharmacological activity.

Aim of study. Determine the antimicrobial and antioxidant activity of fungi.

Materials and methods. The material was alcohol extracts from the fruiting bodies of fungi, obtained by maceration with ethyl alcohol 96% in a ratio of 1:8. The antimicrobial activity of the extracts against *Candida albicans* was determined in a liquid nutrient medium by the method of serial dilutions. Antioxidant activity was determined using the DPPH reagent.

Results and discussion. During the study of antimicrobial activity against *Candida albicans*, the best results were shown by *Hypomyces chrysospermus*, *Cortinarius olivaceofuscus*, *Gymnopilus picreus*, *Cantharellula umbonata*, *Hydnellum ferrugineum*.

The results of antioxidant activity study are shown in the figure. The high level of antioxidant activity can be associated with the content of a large amount of phenolic compounds in the studied fungi.

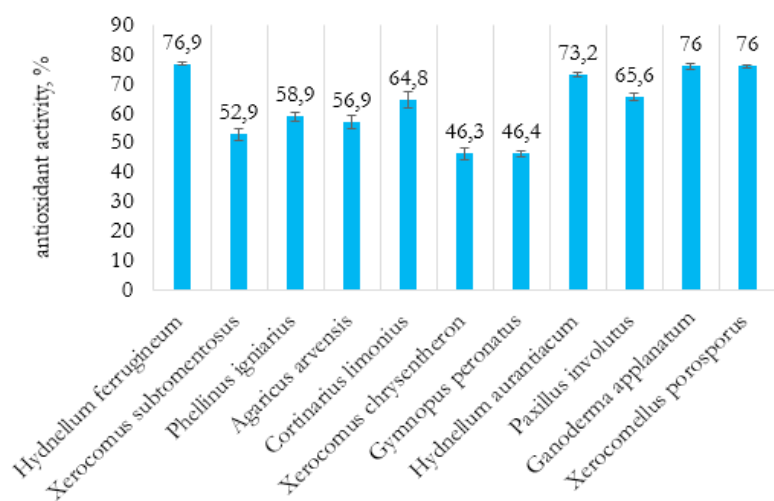


Figure. Antioxidant activity of the studied fungi

Conclusions. The investigated fungi have antimicrobial activity against *Candida albicans*. A number of fungi with high antioxidant activity have been identified.

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ISOQUINOLINE ALKALOIDS FROM *CORYDALIS BRACTEATA*
AND THEIR ACTIVITY ON PLATELET ACTIVATION

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Keywords: *Corydalis bracteata*, secondary metabolites, alkaloids, platelet activation.

Introduction. Cancer is one of the major diseases that cause a large number of deaths. Along with that, cancer is a well-established risk for both arterial and venous thromboembolism and prior studies have shown that cancer-associated thrombosis remains a common complication and leading cause of mortality for cancer patients [1].

Corydalis genus (Papaveraceae) is a rich source of isoquinoline alkaloids. *Corydalis bracteata* (Steph. Ex. Willd.) Pers. is an ephemeral plant with triple dissected leaves and arcuately curved large yellow flower with an ascending spur. According to previous research, alkaloids of *Corydalis* exert significant anti-cancer activity against different cancer cell lines through cell cycle arrest, apoptosis and autophagy, leading to cell death [2]. In current research isolation of alkaloids from *Corydalis bracteata* was carried out. All isolated alkaloids were tested for their influence on platelet activation.

Aim of study. Isolation and structure elucidation of alkaloids from aerial part of *Corydalis bracteata* with further assessment of their activity on platelet activation.

Material and methods. Isolation of individual compounds was carried out from 96% EtOH extract, using open-column chromatography and preparative HPLC. The structures of the individual compounds were elucidated by one- and two-dimensional NMR spectroscopy using a Bruker Avance III 400 MHz NMR spectrometer. The analysis of platelet activation was performed by CytoFlex flow cytometer (Beckman Coulter, Inc.). Platelet α IIb β 3 integrin activation was measured by fibrinogen-Alexa-Fluor 647 binding. Activation of PKA/PKG was monitored by the phosphorylation of VASP using Western blot analysis.

Results and discussion. In current research, two undescribed alkaloids of benzyloisoquinoline and protoberberine types, together with four previously reported protoberberine alkaloids, including coptisine, palmatine, dehydrocorydaline and jatrorrhizine, were isolated from aerial part of *Corydalis bracteata*. All isolated compounds inhibited thrombin-induced platelet activation in 50 μ M and 100 μ M, however differences between their effect were not significant. Also, we evaluated whether inhibition of platelets by isolated alkaloids is mediated by activation of PKA/PKG.

Conclusion. All isolated alkaloids inhibited thrombin-induced platelet activation. However, we found that all isolated compounds do not activate PKA or PKG in human platelets. This is indicating that inhibitory effects of tested alkaloids are not mediated by activation of cyclic nucleotides pathways. Therefore, further research is needed for the elucidation of which pathway is employed by the alkaloids for the inhibitory of platelet activation.

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**CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY
OF ESSENTIAL OILS FROM RESIN OF *COMMIPHORA* SPECIES**

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Introduction. There are several *Commiphora* species belonging to the family Burseraceae in Ethiopia. Ethnobotanical information gathered from local people revealed that the resins of these species enjoy a wide array of traditional uses such as human medicine, to treat maladies of cattle and as insect repellents. In some instances, the fruits, resins and other parts of the plants are used as food additives and as chewing gums. Thus, these resins are of considerable medicinal, cultural and economic significance.

Aim of the Study. The aim of this study was to analyze the chemical composition and evaluated anti-inflammatory activities of four resin oils extracted from botanically identified *Commiphora* species.

Materials and Methods. Essential oils (EOs) were prepared by the hydro-distillation technique from the resins of four *Commiphora* species and analyzed by GC-MS. We investigated the anti-inflammatory effects of EOs in lipopolysaccharide (LPS) stimulated RAW 264.7 macrophages by measuring nitric oxide (NO). The effect in mRNA or protein level after EO treatment were evaluated by RT-PCR and Western blot analysis, respectively.

Results and discussion. Major constituents of EOs were α -copaene (22.71%), β -caryophyllene (28.03%) and β -caryophyllene oxide (13.89%) for *C. sphaerocarpa*; α -pinene (29.1%) for *C. africana*; hexadecane (14.1%) for *C. habessinica* and δ -cadinene (31.5%) for *C. schimperi*. Among four *Commiphora* species, *C. sphaerocarpa* EO demonstrated a significant inhibition of LPS by $27.2 \pm 3.6\%$ at 10 $\mu\text{g/mL}$ and $62.3 \pm 5.2\%$ at 20 $\mu\text{g/mL}$.

C. sphaerocarpa EO inhibited LPS mediated iNOS over expression in both protein and mRNA level with dose dependent manner. It inhibited phosphorylation of ERK1/2, p38, ATF2. The enhanced anti-inflammatory activity of the EO of the plant was due to HO-1 expression by ROS dependent Nrf2 activation in RAW264.7 cells. These findings indicate *C. sphaerocarpa* EO inhibits the pro-inflammatory responses by inhibiting MAPK/ATF2, and triggering ROS/Nrf2/HO-1 signaling.

Conclusions. Therefore, *C. sphaerocarpa* EO could have potential for useful therapeutic candidate preventing and treating inflammatory diseases.

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**SYSTEMATIC REVIEW ON PHYTOCHEMICAL PROFILE AND NUTRACEUTICAL POTENTIAL
OF SPANISH SAGE (*SALVIA HISPANICA* L.), FLAXSEED (*LINUM USITATISSIMUM* L.)
AND PSYLLIUM HUSK (*PLANTAGO OVATA*)**

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Keywords: *Chia seed, flaxseed; psyllium husk; systematic review; bioactive ingredients.*

Introduction. In recent years, people are becoming more aware of and interested in the potential benefits of nutritional support for disease control or prevention [1]. The higher nutritional, phytochemical constituents and healing potential of chia seeds, flax seeds and psyllium husk drives their augmented utilization in industries. These seeds are the source of bioactive substances includes starch, essential fats, vitamins, proteins, dietary fiber, minerals and also contains high amount of phytochemicals could be an effective health promoting factors [2]. In addition to the nutritional advantages, good amount of phytochemicals can play antioxidant role resulting in improving the human health [3]. Moreover, their incorporation in food products especially in the development of gluten-free product can be helpful for the people suffering from celiac disease.

Aim of the Study. Despite the huge interest in these seeds as functional food ingredients. There are very few papers that compare the biological active substances among these products. Thus, this systematic review designed to gather published information and to compare their bioactive ingredients in relation to human health.

Materials and Methods. Comprehensive searches in five databases (Scopus, Web of Science, PubMed, Science Direct and Google Scholar) were carried out for the last five years (2018 to 2023), bestowing to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) procedure. The terms used in searches were (*Salvia hispanica* L., chia seed) AND (*Linum usitatissimum* L., flaxseed) AND (*Plantago ovata*, psyllium husk) AND (Phytochemical profile) AND (nutraceutical potential) for collection of articles, with only articles in English were involved. Transgenic term was excluded. AXIS tool was preferred to assess the quality and risk of bias. The data were then categorized in terms of bioactive ingredients, and its application in human health.

Results and discussion. In total, 121 articles were collected and screened based on the pre-determined inclusion and exclusion criteria. Finally, 10 articles were included in the review. There is convincing evidence that the consumption of these seeds has beneficial effects on human health as they are powerhouse of nutrients. Majority of the publications reported significant findings of bioactive ingredients of chia seeds, flax seeds, psyllium husk grows in different part of the world.

Conclusions. The mentioned seeds are a source of bioactive substance includes essential unsaturated fatty acids, phytochemicals and antioxidants in the human diet. However, the bioactive composition strongly depended on the species of plant from which the seeds were obtained. Incorporation of chia seeds, flax seeds and psyllium husk in food products positive effect on the human health.

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**АКТУАЛЬНОСТЬ РАЗРАБОТКИ СУППОЗИТОРИЕВ
С СУБСТАНЦИЕЙ РАСТИТЕЛЬНОГО ПРОИСХОЖДЕНИЯ**

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Введение. Одной из наиболее актуальных задач фармацевтической технологии сегодня является создание эффективных лекарственных форм с новыми фармакологически активными веществами. Растительные лекарственные препараты обладают широким спектром действия, низким уровнем токсичности и высокой доступностью сырья.

Цель исследования. Обоснование актуальности разработки суппозиториев, содержащих субстанцию растительного происхождения, получаемую из растений рода Амарант (лат. *Amaranthus*) методами изучения литературных данных и маркетингового исследования рынка.

Материалы и методы. Для проведения анализа ассортимента лекарственной формы «Суппозитории» была разработана база данных. Источниками информации служили: Государственный реестр лекарственных средств [1], Регистр лекарственных средств [2]. Актуальность разработки лекарственной формы «суппозитории» изучена с помощью SWOT-анализа [3].

Результаты и обсуждение. Анализ данных литературы показал, что одним из перспективных растений, обладающих широким спектром фармакологического действия (противоопухолевого, противовоспалительного, антиоксидантного и гипохлипидемического) на организм является Амарант, в состав которого входят сквален, токоферолы, фитостеролы и другие биологически активные вещества (БАВ).

Наиболее широкое применение в медицинской практике среди БАВ Амаранта получил сквален, входящий в состав вакцин в качестве адъюванта.

Исследование российского рынка суппозиториев показало, что растительные компоненты занимают только 25% рынка; большая часть суппозиториев предназначена для лечения геморроя или относится к гомеопатическим препаратам. Кажущееся разнообразие действующего спектра препаратов достигается за счет синтетических средств (72%); недостаточно внимания уделено детским лекарственным средствам, они составляют только четверть существующего рынка.

Проведенный SWOT-анализ позволяет сделать вывод, что у данной ассортиментной группы преобладают положительные стороны («сильные стороны» и «возможности»). Сочетание ряда преимуществ суппозиториев как лекарственной формы, а также фармакологического действия БАВ Амаранта позволяют сделать вывод о том, что наиболее перспективная форма лекарственного средства, содержащего субстанцию растительного происхождения, получаемую из растений рода Амарант – это суппозитории.

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