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SYNERGY OF TRADITIONAL AND MODERN APPROACHES TO THE SEARCH AND STUDY OF PROMISING NATURAL-ORIGIN DRUG CANDIDATES (HISTORY, CHALLENGES, SOLUTIONS)

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Until the beginning of the 21st century researchers spent decades searching for new natural sources of medicines using so-called 'traditional classical' chemical, physical-chemical, *in vitro* and *in vivo* methods. The use of this method has allowed the discovery of numerous plants that have become a source of valuable medicinal preparations. However, all of them require significant financial and time investments. Therefore, scientists faced the challenge of rationalizing the process of searching for promising candidates, reducing the time and financial costs of preclinical and clinical research phases, and increasing the efficiency of the final outcome.

Increasingly popular modern method of searching for prospective plant-origin drug candidates is *in silico,* using on-line services and platforms, each of which individually has disadvantages. The most effective is the integration of several service databases.

This allow researchers to identify possible directions for studying the combined effects of isolated substances, thereby reducing the volume of necessary experimental studies *in vitro* and *in vivo*.

The combination of traditional methods of isolation and structure determination with modern *in silico* methods for determining biological activity is confirmed by literature data and our research, in which the combination identified species that are promising for further study.

Keywords: drug candidates, traditional and modern methods, *in silico.*

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Introduction

Until the beginning of the 21st century, both domestic and foreign researchers spent decades searching for new natural sources of medicines using so-called 'traditional classical' methods. The full cycle of research using these methods, from the search for promising candidates to obtaining the drug, took several decades.

Among 'traditional classical' methods is the 'method of affinity' or 'phylogenetic method', where the search for promising sources is conducted among systematically close species within families, genera, and classes. The use of this method has allowed the discovery of numerous plants that have become a source of valuable medicinal preparations. For example, Digitalis, Gentian, and others.

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The 'sieve method' or 'ethnomedicinal approach' involves conducting extensive phytochemical studies of ethnoflora to identify the presence of key biologically active substances. This method, at one point, led to the use of *Andrographis paniculata* in the treatment of dysentery and the isolation of andrographolide – the substance responsible for this particular activity. The 'sieve method' or 'ethnomedicinal approach' involves conducting extensive phytochemical studies of ethnoflora to identify the presence of key biologically active substances. This method has, at some point, led to the use of *Andrographis paniculata* in the treatment of dysentery and the isolation of andrographolide, the substance responsible for this specific activity. Morphine, codeine, papaverine from *Papaver somniferum*, berberine from *Berberis aristata*, and picrozide from *Picrorrhiza kurroa* are also examples of successful implementation of this approach. The 'folk medicine experience' has allowed the discovery of medicinal plants with a millennia-long history of successful use, which in the 20th century became a source for obtaining medicinal preparations. The examples include artemisinin from *Artemisia annua* (antimalarial agent), guggulsterones from *Commiphora mukul* (hypolipidemic agent), boswellic acids from *Boswellia serrata* (anti-inflammatory agent), bacosides from *Bacopa monnieri* (nootropic and memory-enhancing), reserpine from *Rauwolfia serpentina* (antihypertensive), and traditional Chinese medicine (TCM) preparations [1]. Some plants, selected on the basis of different approaches, also have a successful history of being developed into medicines and integrated into medical practice. For example, 'L-Dopa' from *Mucuna prurita* and 'Podophyllotoxin' from *Podophyllum* spp.

Other methods are also known, such as the 'random selection method', 'biodiversity and chemodiversity method', 'life strategy theory', 'ecological approach', 'metabolomics', 'animal behavior observation method', 'chemical defense method' and others [2–11].

Traditional and modern methods of extraction, isolation of individual compounds, and studying the chemical structure of plant-origin drug candidates

The promising species selected during the primary screening stage are further studied using traditional and modern chemical and physicochemical methods. Due to the fact that plants contain complex mixtures of components of various chemical nature and polarity, a number of difficulties arise in the process of extraction, isolation, separation, purification, and identification of biologically active substances. To overcome these challenges, mono-extractants, combinations of extractants with different polarities, chromatographic, and non-chromatographic methods are used [12–14]. In recent decades, methods of enhancing the efficiency and selectivity of isolating target groups/target substances, significantly reducing the duration of processes have been developed. Thus, for the extraction of biologically active substances, methods such as microwave extraction, ultrasound-assisted extraction, accelerated solvent extraction, the use of stationary phases with molecular imprints, hydrophilic interaction chromatography (HILIC), and others have been proposed [15].

Extraction is a necessary process for isolating active ingredients, aiming to maximize the extraction of target chemical compounds while preventing or reducing the dissolution of unwanted accompanying or inert substances. As mentioned earlier, plant extracts are a combination of various types of biologically active compounds with different polarities, which makes their separation, identification, and characterization remain a significant challenge.

The advantages of extraction methods include the simplicity of the method itself and the equipment used, while the disadvantages include incomplete extraction of active ingredients (less than 90%), generally longer process duration, elevated levels of inert substances in extracts (such as fats, pectins, mucilage, proteins, etc.), and labor intensity (double pressing, flushing of cake, etc.).

In classical extraction methods, including maceration, percolation, and reflux extraction, water and organic solvents are commonly used. These methods typically require a large volume of extractant and a long extraction process time. Some modern extraction methods, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), and microwave-assisted extraction (MAE), offer obvious advantages, including economical solvent consumption, shorter extraction times, and higher selectivity.

The obtained extracts constitute a mixture of various compounds that require further separation and purification to obtain an active fraction or pure individual substances. Approaches to separation, including the selection of adsorbents, depend on the physical/chemical differences among the substances in the mixture.

Separation involves the process of isolating substances from plant extracts one by one and purifying them to monomeric compounds using physical and chemical methods.

Classical methods, including solvent extraction, precipitation, crystallization, fractional distillation, salting out, dialysis, paper chromatography, thin-layer chromatography, column chromatography, are methods still widely used in phytochemical research.

On the other hand, modern technologies such as high-performance liquid chromatography (HPLC), flash chromatography, size exclusion chromatography, ultra-performance liquid chromatography (UPLC), ultrafiltration, and high-performance counter-current chromatography (HPCCC), among others, effectively complement and gradually replace traditional methods of extraction, separation, and identification of substances.

For the extraction and identification of biologically active compounds, modern non-chromatographic methods can be utilized, such as immunoassays, employing monoclonal antibodies (MAbs), Fourier-transform infrared spectroscopy (FTIR), and others.

Identification of isolated substances can be performed using traditional methods such as determination of melting point, recording and analysis of electronic spectra in the visible and UV regions, recording and analysis of IR spectra, recording and analysis of proton nuclear magnetic resonance spectra, and conducting enzymatic hydrolysis of substances.

Preparative chromatography has been and remains the primary method for the isolation, separation, and obtaining pure individual substances from complex mixtures. Adsorption column chromatography is widely used for separating substances of different chemical nature. It is often applied in the initial stage of separating substance mixtures due to its simplicity, high capacity, and low cost of adsorbents (silica gel, macroporous resins). Column chromatography on polyamide and silica gel remains a traditional method for separating natural compounds. High-efficiency gas chromatography, with its fast separation and analysis capabilities, makes it a potentially ideal preparative method for separating volatile compounds. Supercritical fluid chromatography combines the advantages of both gas and liquid chromatography, as supercritical fluids have properties such as high solvating power, high diffusivity, and low viscosity, ensuring rapid and efficient separation. High-performance liquid chromatography is a versatile, reliable, and widely used method. The method's advantages include its relative cost-effectiveness, universality in preparative isolation, separation, substance identification, and its applicability as an analytical method for quality control, often referred to as a 'fingerprint' method. Chromatography-mass spectrometry with various detectors is employed for unambiguous identification of total fractions and individual substances obtained after chromatographic separation. This method significantly complements information obtained from other physical and physicochemical methods, particularly allowing the determination of the positions of functional groups in the molecule and assessing their relationships with each other. Nuclear Magnetic Resonance (NMR) spectroscopy is capable of addressing a variety of tasks in the investigation of both multi-component mixtures and individual substances. This includes qualitative and quantitative analysis as well as obtaining “metabolic profiles”. The advantages of the method include the independence on the results obtained by other methods, expressiveness, accuracy, precision, cost-effectiveness (no need for standards, sample preparation), informativeness, and the opportunity to simultaneously obtain information about the structure and content of major and minor substances. Mass spectrometry is another method used in qualitative and quantitative analysis of total complexes and individual substances. The advantages of the method include the ease of operation and equipment maintenance, a small amount of sample required for analysis, high sensitivity, reliability, and the possibility to obtain maximum information about the substance's structure from a single mass spectrum [1, 16, 17].

One of the recent trends in the process of fractional extraction of bioactive compounds from plants involves fractionation combined with parallel determination of biological activity, including the identification of mixtures of synergistic substances that potentiate the action of the target bioactive substance. It is worth noting that synergistic substances often do not exhibit biological activity on their own. However, when present in a mixture with the target substance, they can significantly enhance its pharmacological effects. This approach allows combining chromatographic separation with simultaneous testing of the synergistic mixture and an identified active compound in the original extract. Thus, extracts are tested for synergism, fractionated, active fractions are subjected to synergistic testing again, and the process is repeated until pure bioactive compounds are isolated. Through the combination of a fraction containing known active compounds and the assessment of combined effects, synergistic compounds can be identified [18].

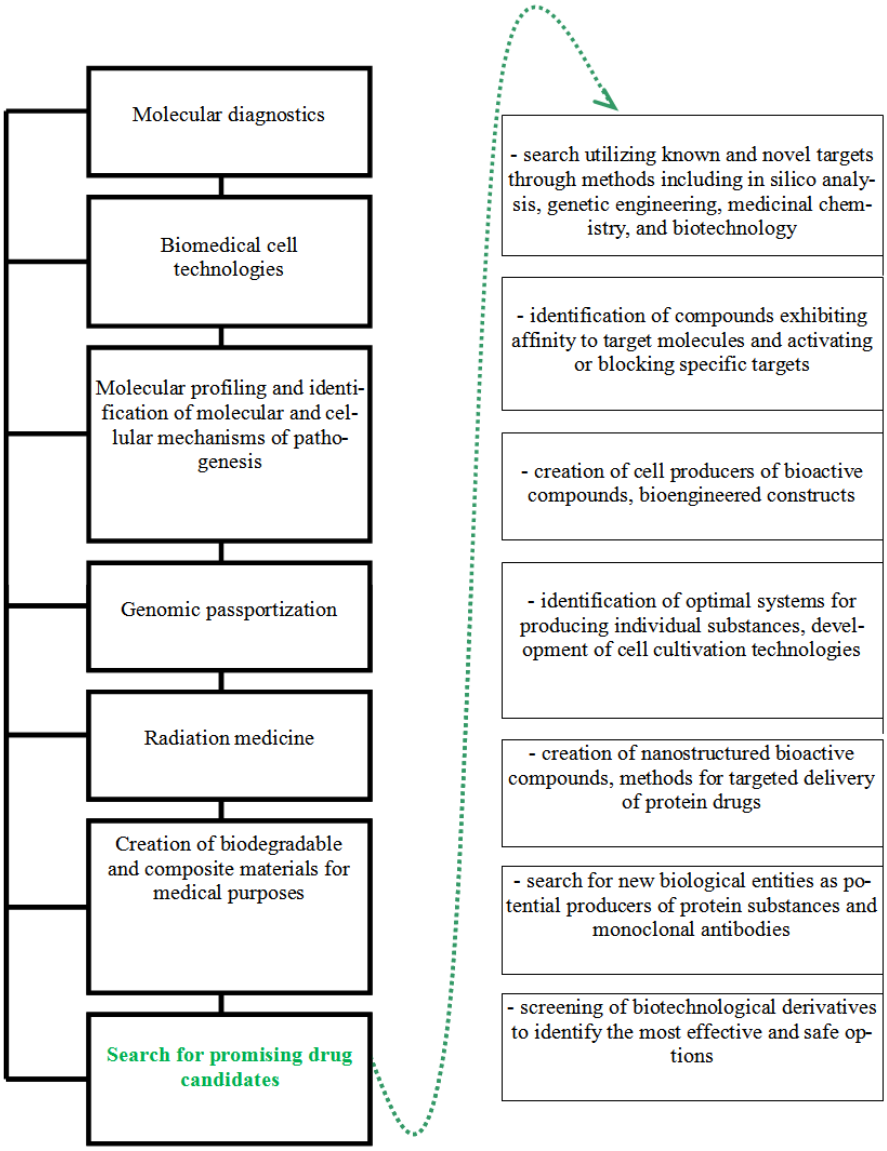
Thus, the combination of chromatographic and non-chromatographic methods is most effectively used for the isolation, separation, and significant acceleration of the purification process of bioactive complexes/compounds; identification of total complexes and purified individual compounds [19]. The obtained information about the

structure of isolated substances, as well as the substances themselves, can subsequently be used for screening pharmacological activity using traditional biological *(in vitro, in vivo)* and / or modern methods *(in silico)*.

The discussed above traditional methods and approaches at the stage of screening prospective candidates require significant financial and time investments. The same applies to subsequent classical stages of pharmacological preclinical and clinical studies *(in vitro, in vivo),* and often the outcome of the work as a whole falls short of the expected results [16, 20]. Therefore, scientists in the past century faced the challenge of rationalizing the process of searching for promising candidates, reducing the time and financial costs of preclinical and clinical research phases, and increasing the efficiency of the final outcome.

Modern methods of searching for promising drug candidates of various origins

One of the priority goals facing Russian science and practice against the backdrop of current sanctions, calls to refuse deliveries of medicinal products to our country is the creation of our own innovative products for the treatment and prevention of socially significant diseases. Achieving this goal is possible through the development of such modern directions as "molecular diagnostics", "molecular profiling and identification of molecular and cellular mechanisms of pathogenesis", "biomedical cell technologies", "genomic profiling", "radiation medicine", "development of biodegradable and composite materials for medical purposes" and "search for promising drug candidates" (Fig.).



Promising directions in the development of medicine and pharmacy in the creation of innovative products

Not only plants can be considered as potentially promising drug candidates, but also plant microbiomes. For example, genomic analysis has revealed that active substances from *Maytenus serrata, Camptotheca acuminata*, species of the genus *Taxus*, on the basis of which anticancer drugs such as "Maytansine", "Paclitaxel" and "Camptothecin" were developed, are produced by microbial endophytes living in the tissues of these plant species. Another example is the work by Helfrich et al., which allowed the identification of hundreds of new biosynthetic gene clusters by analyzing the genome of 224 bacterial strains isolated from *Arabidopsis thaliana* leaves. A combination of bioactivity screening and mass spectrometry was used to select one species for further genomic analysis, leading to the isolation of a PKS-derived trans-acyltransferase and the creation of a natural antibiotic called "Macrobreverin".

Progress in biotechnology allows for the use of plants to produce therapeutic proteins for the manufacturing of drugs and biotechnological products for treating cancer, diabetes, HIV, cystic fibrosis, heart diseases, Alzheimer's disease, and others. They provide an efficient, safe, economical, and rapidly developing platform for the production of therapeutic proteins based on animal cell cultures and microbial fermentation. Moreover, there is minimal risk of plant contamination with animal or human pathogens. The first enzyme approved by the European Medicines Agency for the treatment of Gaucher's disease is taliglucerase alfa (an enzyme obtained from carrots engineered in their cells). Vaccines based on natural substances against the influenza virus are already undergoing clinical trials, while plant lectins are in the developmental stage for producing new anticancer drugs. In the context of the COVID-19 pandemic, there is a need to adapt low-cost technologies for the production of biotechnological natural products against COVID-19. In this context, a promising biopharmaceutical candidate is expected, and vaccines based on virus-like particles (VLPs) have also been announced.

54% of all newly approved drugs have a natural origin (26% are derived from plant sources, 13% are synthesized but their active chromophore originates from plants, and 15% are sourced from other biological origins). Examples of the world's top-selling drugs of natural origin include antibiotics and antifungals: "Erythromycin", "Clarithromycin", "Amoxicillin", "Amphotericin B"; anticancer agents: "Paclitaxel", "Docetaxel", "Camptothecin"; cholesterol-lowering drugs: "Atorvastatin", "Simvastatin", "Lovastatin"; immunosuppressants: "Tacrolimus", "Cyclosporine A"; antihypertensive agents: "Captopril", "Enalapril".

It is widely acknowledged that the beginning of this process was marked by Paul Ehrlich's hypothesis about the existence of chemoreceptors, which was later expanded in the early 20th century by J. Langley, proposing a model of the receptor as a generator of intracellular biological impulses activated by agonists and blocked by antagonists [21, 22]. Thus, the concept of receptors, identification of their structure coupled with advancements in biochemistry, molecular biology, chemistry, and information technologies, has become the driving force behind the rational medication design process, both of natural and synthetic origins. In the 21st century, the timelines from the search for prospective candidates to the release of medication into the market have been reduced to 5–15 years, and the process itself has transformed from 'circumstances aligning' to 'precise calculation'. The information obtained by researchers through traditional methods serves as the foundation for modern databases, software, software complexes, services, platforms on chemical compounds; biological activity modeling, etc. (e.g., PubChem, ChemEMBL, Platinum, Tox21, Molecular Network, Kaggle, Pro Tox, Microcosm, SwissPredict, PASSonline, etc.) [23].

As of the end of 2023, the number of publications in international databases of scientific citation in the field of searching for natural-origin medicinal candidates has been steadily increasing both overall and across specific scientific publications. The significance of plants, along with other sources of natural origin, is underscored by the fact that, according to Newman D. and Cragg G., during the period from 1981 to 2010, 54% of all newly approved drugs had a natural origin [18, 24–26].

Plant-derived preparations possess unique properties compared to synthetic molecules, which present both advantages and challenges in the drug development process. Plants containing complex mixtures of substances are of interest due to the potential for synergistic therapeutic effects of the components in the mixture. They are characterized by a vast diversity of frameworks and structural complexity, having higher molecular mass, a greater number of sp3 carbon and oxygen atoms, but fewer nitrogen and halogen atoms, more hydrogen bond acceptors and donors, lower calculated octanol–water partition coefficients, and greater molecular rigidity compared to synthetic compounds. However, the variability in composition due to, for example, changes in the environment can pose a challenge for drug development and ensuring their stable pharmacological effect. Cultivation, introduction under controlled conditions, cell and tissue culture, and the synthesis of active substance analogs can be potential solutions [16, 24].

The most well-known and increasingly popular modern method of searching for prospective plant-origin drug candidates is computer modeling *(in silico).* According to the U.S. Food and Drug Administration estimates, up to 90% of medications on the U.S. market approved for medical use in recent years have been designed using

computer modeling [15, 25–27]. The capabilities of supercomputers allow comprehensive docking of one billion compounds in less than 24 hours [28].

Despite the significant prospects and undeniable advantages over traditional methods used in pharmaceutical chemistry, pharmacognosy, and related sciences, *in silico* methods have several limitations. Although they imply the execution of computer-based experiments with the generation of valuable and reliable results, mandatory experimental validation of the obtained *in vivo* and/or *in vitro* data is still required. This is because *in silico* methods are imperfect in the sense that they cannot fully account for the diverse impact of drugs on a living organism. Therefore, these methods currently do not allow the partial reduction or complete exclusion of the clinical trial phase, which is known to be the most time-consuming in the search and development of new medicinal candidates [12, 21].

Pharmacological screening was first applied by G. Domagk, who conducted a search for antimicrobial agents among synthesized dyes. He discovered the antimicrobial action of one of them - red streptocid, which marked the beginning of the sulfonamide group of medicines.

Pharmacological screening involves the selection of prospective candidates of natural origin, chemical, or biological synthesis using various methods of actual or virtual potential pharmacological activity. A significant advantage of modern methods is that researchers often do not need to have the physically tested substance. Additionally, they are not methodologically restricted within the framework of searching for prospective species / candidates, for example, within one genus, family, or one group of derivatives, etc.

At present, screening of biological activity includes virtual screening, synthesis of substances with a specified structure, and panel screening.

Virtual screening is based on the expectation that the biological activity of substances is directly related to the structure of both existing and yet-to-be-existing compounds. It involves the selection of compounds based on their effectiveness, selectivity, specificity, optimal absorption, distribution, metabolism, elimination, pharmacokinetic properties, etc. For example, derivatives of 2–8 benzylpyrimidines are used to create antihistamine drugs; quinoline derivatives – anti-tuberculosis agents; derivatives of 1,5-oxadiazole – antihypertensive agents, and so forth.

Virtual screening allows the development of various models: biological, pharmacological, economic, and statistical. These methods provide the opportunity to conduct preclinical trials, exploring biological activity, studying disease models, and examining substance properties. Predicting the activity direction of compounds is of particular importance, as modern pharmacology deals with more than two thousand types of biological activity. Statistical and economic models enable the testing of drug effectiveness on large numbers of patients, calculate costs, profitability, potential revenue, and design clinical trials on healthy volunteers. All of this significantly reduces the time and resources spent on creating a new medicine and bringing it to market, and the use of these methods is a necessary condition for the successful implementation of research programs in the development of new medications [29–32].

Virtual screening involves the preparation of a biomolecular target model (charge distribution on atoms); preparation of databases of organic compound structures (calculation of physicochemical properties, modeling of spatial structure, calculation of atomic charges); preprocessing of databases (removal of structures based on physicochemical criteria such as lipophilicity, molecular weight, predicted toxicity, etc.); molecular docking - a method of molecular modeling aimed at testing the successful activity of active compounds against potential gene targets, based on predicting the most favorable position of molecules in space relative to each other; post-processing of generated databases of potential ligands using QSAR models, resulting in a focused library of potential ligands for a given biomolecular target. QSAR's task is to predict activity based on the compound's structure, construct chemical structures with specified activity values, and then synthesize them. The structural formula is represented in mathematical form, which can describe both the biological activity and any compound property. The QSAR model represents a linear "property-structure" dependence.

In addition to ligand-based virtual screening, there is virtual screening of biological activity using pharmacophores – structural elements or fragments of a molecule that provide pharmacological activity of a compound. A pharmacophore model consists of a set of points in space with specific physicochemical properties, binding sites, and distances between them. Virtual screening using a pharmacophore model involves selecting molecules that meet the requirements of this model regarding functional groups and distances between them.

After selecting the compound structure, synthesis of the substance is carried out, followed by investigation of its biological activity using panel screening on a biochip - a matrix onto which biological macromolecules, i.e., biomolecular targets (DNA, proteins including enzymes, cells), capable of selectively binding substances contained in the analyzed solution, are applied.

Most researchers highlight that the advantages of *in silico* screening include a high level of standardization, low cost, minimal amounts of the substance under investigation, the ability to identify structure/activity correlations, and generally no need for animal testing. However, drawbacks include a high probability of errors, the inability to account for the diverse effects of substances on living systems, and testing conducted only at one dose [33–35].

Today, researchers, clinicians, and students have access to several ready-made solutions, platforms, online services, and docking programs for conducting *in silico* research, training, and studying model animals and patients. For example: «HumMod», a Windows-based mathematical model that simulates human physiological processes. It accurately predicts both qualitative and quantitative changes in clinical and experimental responses. It can be used in science, medicine, and education. «Oncosimulator project», an integrated software system designed for modeling, researching, aiding in the selection of chemotherapy regimens for individual patients, and developing and interpreting clinicogenomic trials. It serves as a tool for training physicians, researchers, and interested patients. «SwissTargetPrediction», an online tool designed to predict the most likely protein targets of small molecules based on similarity principles, using reverse screening. It contains a database of 376342 molecules and 3068 macromolecular targets, achieving a high level of predictive efficiency. «PASS" on way2drugs, a service allowing the selection of promising substances for synthesis by determining directions for testing their biological activity. The prognostic model underlying this service is built on reliable, repeatedly verified data, achieving a high level of effectiveness, reliability, and alignment with widely accepted concepts regarding the mechanisms of action and possible biological effects of known and new compounds [36, 37].

Among the drawbacks of some of the mentioned services is that they are not intended for modeling the biological activity of compounds, have a narrow focus, and so on. Another limitation is that they cannot be applied to predict the combined biological activity of two or more chemical compounds. In most cases, the biological effects of a group of drug candidates are considered independently, and predictions are made separately for each compound. In practice, such an approach does not offer any fundamental advantages over the traditional experimental approach, as combined biological effects must be studied experimentally. One solution to these issues could be the integration of multiple databases/services, allowing the identification of possible directions for exploring combined effects, thereby reducing the volume of experimental research [37].

Researchers also have access to several molecular modeling programs for rigid and flexible docking: DOCK, AutoDock, e-Hits, FlexX, LigandFit, FRED, Glide, GOLD, QXP, Surflex-Dock, and others, as well as databases such as String, IntAct, iHOP, BioGRID, MIPS. Molecular docking involves accurate prediction of the orientation and biologically active conformations of two interacting molecules and evaluation of the tightness of their complex. The most popular and rapidly developing programs are Dock, AutoDock, FlexX, and Glide. The comparison of the accuracy of ligand-receptor interaction predictions between different software products shows the following decrease in accuracy: Glide 82%, Surflex 75%, FlexX 58%, GOLD 78%. Problems that may arise when working with docking programs include: the accuracy of binding structure, scoring function, involvement of water molecules, receptor flexibility, and ligand conformations.

The practice of using modern and traditional methods in the search for promising natural medicinal candidates

Chinese scientists have achieved significant success in applying the methods, approaches, and services discussed above. They have identified active compounds from well-known traditional Chinese medicine (TCM) preparations, elucidated molecular targets, and signaling pathways in various pathologies. For example, quercetin, kaempferol, β-sitosterol from "Huangqi Guizhi Wuwu" for rheumatoid arthritis [38]; naringenin, kaempferol, formononetin, quercetin, isoflavone, 7-methoxy-2-methyl from "Dayuanyin"; baicalein, quercetin from "Huashi Baidu"; luteolin, ursolic acid, quercetin, and rutin from "Jinghua-Qingan" and "XuanFei-BaiDu", "Xuebizin", "Lianhua-Qingwen", "9* HuaShi-BaiDu", "Qingfei-Paidu" for COVID-19 [39–41]; active ingredients in "Shuxuening" that suppress inflammation, regulate the degree of oxidative stress, minimize neuronal cell death in brain tissue, thereby protecting it in ischemic stroke [42]; quercetin, luteolin, naringenin in "Yanghe", which have antitumor properties, molecular synergy in HER2-positive breast cancer [43]; α-sitosterol, propylene glycol monoleate, campesterol, and 25-oxo-27-norcholesterol from sorghum bicolor, which reduce the severity of type 2 diabetes by activating receptor signaling pathways activated by PPAR [44]; several active compounds from *Tinospora sinensis*, which significantly influence the expression of the PI3K and Akt protein through a regulatory network, multiple targets, and pathways, and therefore may prevent and treat Alzheimer's disease [45]; 48 biologically active compounds in "Shaoyao-gancao", 30 targets, and multiple pathways associated with Parkinson's disease [46].

The successful application of modern approaches to predicting pharmacological activity is also reflected in other studies [2, 47–51].

As indicated by the data presented above, in most conducted *in silico* studies, phenolic compounds have been identified and investigated as active substances, mainly flavonoids, isoflavonoids, chalcones, anthocyanins, and catechin derivatives. Therefore, in our opinion, it is advisable to search for promising drug candidates among species that predominantly accumulate phenolic compounds.

One of the promising genera in terms of containing phenolic compounds is the genus *Empetrum* L., where they constitute the predominant group. According to the literature and the results of our research, water extracts of crowberry restore the activity of cellular antioxidant enzymatic systems, inhibit lipid peroxidation, reducing the production of malondialdehyde. This suggests that the antioxidant potential of representatives of the genus can be used to create medicinal agents for the therapy of pathological conditions where oxidative stress is involved in the pathogenesis (neurodegenerative diseases, hypoxic conditions of various etiologies, vascular and immune disorders, degenerative changes in the hepatobiliary system, oncological diseases, etc.) [2].

Another promising species in terms of containing polyphenolic compounds is *Iris lactea*. The most characteristic compounds for this species are flavonoids (C-glycoside flavones – embinin and its derivatives), isoflavonoids – irison B, tectorigenin, etc., xanthones – iriflophenone and its derivatives, mangiferin, bellidifolin, etc. The application of *Iris lactea* in traditional and modern medicine is mainly based on the presence of isoflavonoids. A wide spectrum of effect of the extracts has been established (anti-inflammatory, antimicrobial, antioxidant, anti-hypoxic, cardiotonic, antiviral, immunostimulating, cytotoxic), which makes *I. lactea* a promising object for further research [52].

Ononis arvensis, from the aerial part of which we have isolated and characterized isoflavonoids and flavonoids, is another promising species for further study. According to the literature, several well-known pharmacological effects are associated with polyphenolic compounds, including diuretic, cholagogue, analgesic, and anti-hypoxic effects [53].

Various morphological organs (seeds, leaves, roots) of the widely distributed species of burdock, *Arctium lappa* and *Artium tomentosum*, found in Russia, are rich in hydroxycinnamic acids and lignans, according to our data. The presence of these compounds is associated with many experimentally confirmed pharmacological effects [54, 55].

Using the PASS program (v.2020), we have identified the likely spectrum of pharmacological activity of individual substances, including those isolated for the first time from *Solidago canadensis*, *Rubus chamaemorus* – species known for their rich composition of phenolic compounds. In addition to confirming known pharmacological properties, we have identified previously undescribed ones and determined possible vectors for their use. This, in our view, could serve as a basis for further study of these species as a potential source of medicinal candidates [53, 56, 57].

The results of *in silico* screening contribute to various databases, facilitating the work of researchers in the future [58–65]. One such recently created database for identifying drug candidate for COVID-19 is "CoronaDB-AI" [66].

Thus, *in silico* tools enable the implementation of various tasks ranging from training, selecting drugs for specific patients to screening prospective groups of compounds for further detailed investigation. They also aid in formulating requirements for conducting experimental studies on biological models (*in vivo, in vitro).*

Conclusion

There are still many understudied or unexplored plants in the world's flora that could serve as promising medicinal candidates for developing highly effective and safe drugs. Information about isolated individual compounds could become a valuable source of data for modern databases, on-line services and platforms.

Today it is crucial to combine modern *in silico* methods with traditional approaches for the search, investigation, determination of composition and structure of substances, and assessment of the biological activity of prospective drug candidates.

The literature data on *in silico* studies of plant preparations and individual substances demonstrate that phenolic compounds, primarily flavonoids, isoflavonoids, chalcones, anthocyanins, hydroxycinnamic acids, and lignans, are often active substances with a broad spectrum of action. Our own research corroborates these findings, identifying promising species for further study. These include representatives from various plant families, such as species of the genus *Empetrum* L. and *Arctium* L.*, Solidago canadensis, Rubus chamaemorus, Iris lactea*, and *Ononis arvensis.* All of these species possess high antioxidant potential, which can be harnessed for the development of medicinal products aimed at treating pathological conditions where oxidative stress plays an important role

(neurodegenerative diseases, hypoxic conditions of various etiologies, vascular and immune disorders, degenerative changes in the hepatobiliary system, oncological diseases, etc.).

Researchers have access to several platforms for conducting *in silico* studies, each of which has its own set of drawbacks. One such drawback is the inapplicability for prediction of the combined biological activity of multiple compounds. One potential solution to this problem could be the integration of multiple databases from well-developed services, such as way2pass and SwissPredict. This integration would allow researchers to identify possible directions for exploring the combined effects of substances, thereby reducing the volume of required experimental research *in vitro* and *in vivo.*

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Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

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