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Use of Metabolomic Approaches in Analysis of Medicinal Plants and Phytopreparations (Review)

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Abstract

Introduction. The purpose of the analytical review is to summarize the data of modern scientific literature on the directions and possibilities of using the approaches of metabolomics in the analysis of medicinal plants, plant raw materials and herbal drugs.

Text. Analysis of literature data showed that metabolomic approaches have great potential in the field of quality control of multicomponent phytopreparations and biologically active additives, detection of falsifications of rare and expensive plant materials, chemosystematics of medicinal plants, study of the mechanisms of action and toxicity of medicinal plants, etc.

Conclusion. Metabolic analysis can become an effective analytical platform both for phytochemical research of plant raw materials and for regular activities to control the quality of plant material and phytopreparations.

Keywords: metabolomics, phytopreparations, medicinal plants, quality control, phytochemistry.

Conflict of interest: no conflict of interest.

Contribution of the authors. Anastasiia A. Orlova, Jovana Strugar, Maria N. Povydysh – selection of materials, preparation of the text of the article; Oksana Yu. Shtark, Vladimir A. Zhukov, Vladimir G. Luzhanin – discussion of the results, editing the text of the article.

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Использование подходов метаболомики в анализе лекарственных растений и фитопрепаратов (обзор)

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Резюме

Введение. Целью аналитического обзора является обобщение данных современной научной литературы о направлениях и возможностях использования подходов метаболомики в анализе лекарственных растений, растительного сырья и фитопрепаратов.

Текст. Анализ литературных данных показал, что метаболомные подходы имеют большой потенциал в области контроля качества многокомпонентных фитопрепаратов и биологически активных добавок (БАД), выявления фальсификаций редкого и дорогостоящего растительного сырья, хемосистематики лекарственных растений, изучения механизмов действия и токсичности лекарственных растений и т. д.

Заключение. Метаболомный анализ может стать эффективной аналитической платформой как для фитохимического исследования растительного сырья, так и для регулярных мероприятий по контролю качества растительного материала и фитопрепаратов.

Ключевые слова: метаболомика, фитопрепараты, лекарственные растения, контроль качества, фитохимия.

Конфликт интересов: конфликта интересов нет.

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INTRODUCTION

Concept "metabolom" was first offered in 1998 by Steven Oliver for designation of low molecular compounds synthesized by the body [1]. Plant metabolism is the combination of all primary and secondary metabolites and can be considered as the result of implementation of genetic information, “binding link” between the genotype and phenotype [2, 3]. In 2002, Oliver Fien introduced concept “metabolomics” for description of the integral analysis including identification and quantification of all metabolisms in the body [4].

Plant metabolomics is commonly determined as the total qualitative and quantitative analysis of the metabolites contained in the biological system in certain conditions [5]. The metabolomic methodology may be conditionally divided into targeted or untargeted metabolomics. The first one assumes the targeted search and, as a rule, quantitative analysis of a few substances known a priori. Untargeted metabolomis is the comprehensive analysis of all measured metabolites including unknown ones [6–8].

The main approaches related to the metabolome studies are given below:

1. *Metabonomics* (from Greek "*meta*" – change, and "*nomos*" – a set of rules or patterns) – is commonly used for investigation of non-plant organisms and provides a quantification of endogenous metabolites which contents is dynamically changed in the live system in response to pathophysiological stimuli or genetic modification. Tissues and biological fluids are usually used for the analysis. It may be used for detection of mechanisms of action of herbal products [9–11].
2. *Metabolite profiling*. Identification and quantification of the complex of metabolites connected by the common metabolic pathway or affinity of the chemical structure. Metabolite profile can be determined as the set of all metabolites and/or their derivatives (identified or unknown) found with the certain analysis method [12, 13].
3. *Fingerprinting*. A prompt and high performance method aimed to obtain metabolite profile in aggregated extracts. Usually, the method does not identify and quantify metabolites [5, 14].
4. *Targeted analysis of metabolites*. Qualitative and quantitative analysis of one or several pre-determined metabolites related to a certain metabolic reaction. Such approach is based on the metabolic extraction, separation and identification of metabolites [15, 16].

Modern English scientific literature contains dozens of the reviews devoted both to general aspects of plant metabolomics [17–20], and private cases of its use in the analysis of medicinal plants [21–24]. The rapidly developing trend has also been reflected in a number of practical guidelines and protocols [25-28]. Unfortunately, there are only few articles in Russian [29–32].

The aim of the analytical review is to summarize modern scientific literature data on possibilities to use metabolomic approaches in the analysis of medicinal herbs and herbal preparations.

RESULTS

Specialists in metabolomics worldwide emphasize that all stages of metabolomic studies should be standardized that will allow to compare the data correctly and also to create the public database which, combined with proteomny and transcriptomy databases, will be used in functional genomics and system biology [33–37].

Generally, the flow chart of plant metabolomic analysis includes:

1. Sample preparation (preparation of plants raw materials, drying, extraction, derivatization, purification).
2. Sample analysis based on different types of mass spectrometry (GLC-MS, HPLC-MS) or nuclear magnetic resonance spectroscopy.
3. Processing of the obtained results (data normalization, scaling, statistical modeling, identification of chromatographic peaks).
4. Result interpretation.
5. In systemic biology, a hypothesis formulation and its experimental test follows.

Nowadays, there is a trend of generation of integrated protocols for the most widespread analytical methods. So, metabolomic protocols based on LC-MS [38], HPLC-MS [39], nuclear magnetic resonance [19, 21] etc. were formalized [40, 41]. During the last decade, a number of the practical guidances and reviews intended for standardization of key steps in investigations of plant metabolism [25, 26, 42, 43] were published. The stages of sample preparation while processing plant objects are detailed in the work by H. K. Kim and R. Verpoorte (2010). The impact on the result of investigations of such factors as preparation time, selection of plant organ, particularities of drying of plant raw materials, etc. is described [44].

The key problem of modern metabolomics is the work with numerous separate data [45, 46]. There are some reviews describing the existing electronic databases for plant metabolomics including mass spectrum bases for various groups of metabolites, bases of structures and chemical characteristics of natural compounds, databases of plant metabolic pathways, databases of components of traditional medicinal plants [47–49]. The list of the most widespread databases is given in figure 1.

USE OF METABOLOMICS IN RESEARCH OF MEDICINAL HERBS

Analysis of multicomponent herbal preparations and dietary supplements

For standardization and quality control of multicomponent extracts from plant raw materials and drugs, metabolomic approaches based mainly on

Mass Spectrum Databases	Bases of structures and chemical characteristics of natural compounds	Plant metabolic pathway data	Analysis data of metabolic profiles of plants
MassBank - http://www.massbank.jp/	Chemical Abstract Service (CAS) - http://www.cas.org/	KEGG - https://www.genome.jp /kegg/pathway.html	PlantMetabolomics.org - http://www.plantmetabolomics.org
NIST - http://www.sisweb.com /software /ms/nist.htm	PubChem database in NCBI - http://pubchem.ncbi.nlm.nih.gov	UniPathway - http://www.unipathway.org/	Medicinal Plant Metabolomics Resource - http://metnetdb.org /mpmr_public/
Golm Metabolome Database (GMD) - http://gmd.mpimp-golm.mpg.de/Default.aspx	ChemSpider - http://www.chemspider.com/	SMPDB - http://www.smpdb.ca	MeKO database - http://prime.psc.riken.jp/meko/
Spectral Database for Organic Compounds (SDBS) - http://riodb01.ibase.aist.go.jp/sdbs /cgi-bin/cre_index.cgi?langDeng	KEGG compound - http://www.genome.jp/kegg /compound/	iPath - http://pathways.embl.de	KOMICMarket - http://webs2.kazusa.or.jp/komics/
MassBase - http://webs2.kazusa.or.jp /massbase/	Plant Metabolome Database (PMDB) - http://www.sastra.edu /scbt/pmdb/	PlantCyc - http://www.plantcyc.org/	McGill MetabolomeDatabase - http://metabolomics.mcgill.ca/
MetabolomeExpress and MetaboLights - https://www.metabolome-express.org/	Manchester Metabolomics Database (MMD) - http://dbkgroup.org/MMD/	BioCyc - http://biocyc.org/	
METLIN - http://metlin.scripps.edu			

Figure 1. The most useful resources in plant metabolomics research

a mass-spectrometry and ^{1}H NMR are successfully used [50, 51]

Thus, the comparative composition evaluation and further statistical processing with the main component method were performed using *Artemisia afra* and *A. annua* metabolites, and Artemisia nutritional supplement which, according to the manufacturer's description is produced on the basis of *A. afra* extract and has the antimalarial effect related to the high artemizin content, was performed with HLPC-MS and NMR methods. As a result of the analysis, no artemizinin traces in *A. afra* sample and in plant material of the capsules were found. Thus, it can be used as a reliable method for quality control of dietary supplements of plant origin and identification of counterfeits [52]. The quality control of traditional and Ayurvedic medicines is also a considerable problem in modern pharmacy as a chemical composition of these multi-component products is not always understood completely. Metabolic profiling of such structures and investigation of their standardization methods help to obtain the conclusive research evidence and to increase their recognition by scientific community and consumers [53, 17].

Identification of counterfeits

If a product contains rare or expensive raw materials, counterfeits are often present which identification in quality control represents an important task. Metabolic profiling with the multidimensional analysis of ^{1}H NMR-spectra ranges allows to identify closely related types and identify unfavorable impurities. Thus, the possibility to differentiate poisonous bark Strychnos nux-vomica (so-called "false angostura") from bark of a tropical angostur tree (*Angostura trifoliata*) used as a tonic or flavoring additive of bitter alcoholic beverages was shown Brucine, loganin and fatty acids appeared to be diagnostic compounds [54].

It is shown that ^{1}H NMR spectroscopy without pre-separation of metabolites combined with the multi-dimensional statistical analysis allows to differentiate effectively Echinacea samples (*E. purpurea*, *E. pallida* and *E. angustifolia*) which are quite often substituted on the European pharmaceutical market [55].

Hemosystematics of medicinal herbs

The targeted and untargeted approaches to a profiling test metabolites used for identification of plant specifics and generation of botanical systems [56, 57]. For identification of related *Panax* species, the untargeted screening of secondary metabolites with the method of a highly performance chromatography coupled with mass spectrometry was performed. Three biomarkers – chikusetsusaponin IVa, ginsenoide Rf and ginsenoside Rc as the most specific biomarkers for *Panax ginseng* were determined [58]. Using the targeted metabolomic analysis, variability of 10 marker phenolic compounds and iridoids (nepetalactones) in 12 Nepeta species was investigated with the ultra high performance liquid chromatography. As for medicinal herbs, the identification of hemomarkers has the practical importance and can be used for differentiation of morphologically related species [59].

Investigation of toxicity and biological activity mechanisms of medicinal plants

The establishment of mechanisms of toxic action of natural compounds helps to determine safe methods for their use in medical practice [60]. Zhang et al. (2006) used metabolomic methods for the toxicity study of aristolochic acid contained in *Aristolochia* plants. The analysis of biochemical urine values of rats with ^{1}H NMR method after introduction of aristolochic acid and several toxins with the known mechanisms of action allowed to reveal the damage of proximal tubules and papillary nodes of the urinary system, negligible liver damage exposed to aristolochic acid and its accumulative action. These results were confirmed with the common clinical biochemical methods [61].

Recently, several studies on metabolism pathways of plant extracts and their main components in the body in *in vivo* experiments have been conducted. The proteomic and a metabolomic approaches are effective strategies for understanding of drug mechanisms of action on infectious agents [62]. Thus, for the search of new drugs for *Mycobacterium tuberculosis* infection, the metabolic profile of *M. tuberculosis* culture treated or not treated with medicinal herb extracts was determined. The metabolites which differ in treated and untreated cultures allow to find targets and mechanisms of action of natural compounds [56].

Search of plants – sources of perspective metabolites. "Reverse" pharmacognosy

The new approach for the search of sources of prospective natural compounds was called "reverse pharmacognosy" Conventional pharmacognosy uses

plants for detection of new biologically active compounds within, whereas the reverse pharmacognosy uses data on natural compounds for the search of new properties of medicinal herbs [63]. The objective of reverse pharmacognosy is the search of new biological targets for natural compounds by virtual or real screening and identification of the natural resources containing active molecules [64]. Such approach uses rich knowledge of ethnopharmacology along with the modern technical opportunities including metabolomics and *in silico* methods. Databases of natural compound structures, biological targets, ethnopharmacological knowledge and resources for virtual screening should be available for effective use of the approach [49].

Pharmaceutical industry lacks candidate drugs and drug repositioning, i.e. establishment of new types of administration for the existing drugs, as for herbal preparations, it may be successfully implemented by the combined approaches of direct and reverse pharmacognosy [65]. As the majority of natural compounds interacting with various targets show pleiotropic (multiple) effects, computing methods are integral for the search of new drugs of natural origin. As evidenced by investigation of pleiotropic therapeutic effects of 50 medicinal plants of traditional Indian medicine, the efficiency of computer programs PASS and PharmaExpert was shown for the analysis of biological activity spectra of herbal components, both alone and in combinations. The approaches *in silico* including virtual screening have allowed to identify new targets for tested natural molecules that go beyond conventional use of corresponding medicinal plants [66, 67].

Targeted change of the synthesis of secondary metabolites

It is established that interaction with other organisms has a direct effect on biochemical plant reactions: body protection reactions against a pathogen or a parasite, or signaling cascades targeted to mutually beneficial relations with a symbiont [68]. H. C. Choi et al. (2006) used tobacco leaves infected with the tobacco mosaic virus as the test model and revealed that the infection led to the increase of phenylpropanoids involved in the enforcement of the cell wall, as well as, caffeoylqunic acid involved in protective body reactions [69]. Arbuscular mycorrhiza is the mutually advantageous symbiosis of plants with *Glomeromycota* mushrooms developing in plant roots. It is shown that mycorrhized plants produce a larger quantity of therapeutically essential compounds such as phytoestrogen, alkaloids, furanocoumarins and pterocarpans, as well as antioxidants and the essential oils [70]. Being mycorrhized in peas leaves (*Pisum sativum* L.) at late development stages, the pathways of monobactame and steroid biosynthesis, and porphyrine, and a chlorophyll metabolism were involved. Mycorrhization caused some delay in changes of metabolomic leaves related to the increase of plant age [71].

Metabolomic approaches can also be useful in derivation of new genetic lines with the increased production of target components. So, 26 germinal lines of *Scutellaria baicalensis* with various phytochemical profiles were derived in vitro. Extracts of the obtained samples contained more than 2000 compounds. Untargeted metabolomic approach was used for the establishment of correlation between the chemical composition and drug potential. As a result of the research, the selection criteria were offered for the most perspective lines on the basis of the antioxidant potential, growth rate, contents of baicalin, melatonin and vogonin [72].

Production of drugs from plant cell cultures has a number of the advantages associated with their possible use of various biotic and abiotic elicitors and the best control for accumulation of biologically active agents. The methods of HPLC-MS and NMR spectroscopy identified secondary metabolites in cell cultures *Panax japonicus*, *Tribulus terrestris* and *Dioscorea deltoidea*. The prediction of biological activity ranges of the compounds using PASS program has also shown prospectives of experimental action mechanisms related to with manifestation of anti-hypoxemic effect for each of the compounds. Software PharmaExpert has allowed to analyze the possible synergetic or additive effects of metabolite combinations related to the type of activity. The prediction *in silico* was confirmed in experimental studies of anti-hypoxemic effect of the extracts on animals [73].

Effect of preparation conditions on a metabolomic profile

It is known that manifestation of seasonal variability in qualitative and quantitative composition of secondary metabolites may affect quality of the raw

materials used for production of herbal preparations and dietary supplements. The studies of secondary metabolite profiles can be used to examine variability of the chemical composition depending on preparation time, drying and primary treatment conditions, and morphological group of plant raw materials. Scognamiglio et al. (2014) showed seasonal structure changes of metabolites in extracts of *Phillyrea angustifolia* leaves collected from April to March with the method of ^{1}H NMR profiling and the multidimensional data analysis. During the study, quantitative changes of iridoid contents within a year and various degree of phytotoxicity in relation to test plant Triticum ovatum were established [74].

CONCLUSION

The literature review has shown that the use of metabolomics for plant objects represents a convenient and effective tool to identification of pharmacologically active molecules and for the solution of a number of theoretical and applied problems. However, it should be noted that the results of similar studies are still hardly comparable due to high variability of methodological and technical approaches to their implementation. Based on that, the development of the standard unified analysis procedures and generation of complex databases for evolving informative metabolomics represents the relevant expansion in this field.

Nowadays, the use of metabolomics while working with medicinal herbs is limited in most cases to metabolomic profiling for identification of new natural compounds. The literature analysis has shown that metabolomic approaches have also a high potential in the routine and express analysis of herbal preparations and dietary supplements, identification of counterfeits of rare and expensive herbal raw materials, hemosystematics examining mechanisms of action and toxicity of medicinal herbs.

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