ABSTRACT

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on specialty 6D072100 – "Chemical technology of organic compounds"

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"Development of Comprehensive Processing Technology of Medicinal and Pharmacopeial plants of the Genus Polygonum"

This work is devoted to the development of technologies of the production of dry extracts from some species of pharmacopoeial plants of the genus *Polygonum* L., what has cannabinoid and opiod biological activity.

The actuality of the research topic

There is the need for new medicines while level of industrial emissions and environmental degradation have increased. One of the ways to solve this problem is the using of herbal medicines. Since Kazakhstan is a rich region for various types of plants (more than 6,000 species), its poor knowledge leads to the fact that significant amounts of medicinal raw materials and phytopreparations are imported from the CIS countries and another countries.

Currently, there is a capacity for health and the pharmaceutical industry of the Republic of Kazakhstan in new, effective medicinal drugs from plants of the local flora. A successful solution of this problem will contribute to the implementation of the Program for the Development of the Pharmaceutical and Medical Industry of the Republic of Kazakhstan until 2025.

The Kazakh government has set its sights on the saturation of the local pharmaceutical market with own-produced drugs and, primarily, on the basis of local (regional) plant resources. In addition, the World Health Organization (WHO) recognizes the use of plants as raw materials for pharmacy as one of the highest priorities.

The Republic of Kazakhstan possesses the richest plant resources, but only a small part of them are used by medicine and the national economy. Currently, world medicine uses more than 30% of medicines are made from plants, while for the treatment of a number of diseases, many herbal remedies are indispensable.

In this regard, wild-growing plants of the family *Polygonaceae* Juss. has great theoretical and practical interest. They are widely distributed in Kazakhstan. The studied plants of this family have different types of biological activities and are used to treat various types of inflammation, tumors and other diseases.

The study of the chemical composition of new wild-growing species of the family *Polygonaceae* Juss., the development of ways of the isolation of potentially biologically active substances, the study of biological activity and the development of new phytopreparations from wild Kazakh plants is relevant and has social importance, as it

will expand the range of medicines of the Republic of Kazakhstan, what will be produced from local renewable plant materials.

The aim of the work is to determine the optimal parameters of technology for the isolation of substances from plants of the genus *Polygonum* L., and their standardization.

There were the following **tasks**:

1. To study the quality of the studied plant species (*P. hydropiper, P. aviculare, P. minus*);

2. To establish the qualitative and quantitative composition of the main groups of biologically active substances (BAS) in the studied plant species;

3. Develop and establish the optimal technological parameters for obtaining dry extracts from the plants;

4. To develop laboratory technology of the production of crude extracts;

5. To standardize substances, develop and draw up optimal ways for the separation of their BAS complexes into individual compounds and identify the isolated substances.

6. To study new types of activity of isolated substances and fractions from dry extracts for their using in medicine.

The object of study is the aerial parts of Kazakh pharmacopoeial species of plants of the genus *Polygonum* L. of the *Polygonaceae* Juss family (pharmaceutical samples): *Polygonum hydropiper* L., *Polygonum aviculare* L., *Polygonum minus* Huds.

The subject of the research is the aerial parts of three pharmacopoeial Kazakh species according to standard indicators of their quality (pharmacopoeial articles) and quantitative content of active and associated substances, the choice of optimal extractants, the ratio of raw materials: solvent, extraction method and extraction time. Biological screening of the received potential phytopreparations was carried out on test samples for their further recommendation for medical uses.

Research methods. The main physico-chemical research methods used in the work were UV and IR spectroscopy, as well as the structures of all the selected substances were identified by one-dimensional (^{1}H and ^{13}C NMR, DEPT), two-dimensional (HMQC, HMBC, COZY), mass and chromatography-mass spectrometry.

Basic provisions to be protected:

- a comparative analysis of the qualitative and quantitative content of the main groups of biologically active substances of aerial parts of three wild species: *P. hydropiper, P. aviculare, P. minus* growing in the Republic of Kazakhstan;

- description of the technology for producing dry crude extracts from the aerial parts of the plants of the genus *Polygonum* L.;

- separation and isolation of crude substances into individual components using spectral identification;

- results of biological tests of isolated fractions and individual substances for medical uses.

Scientific novelty

1. Original methods of spectrometric quantitative determination of flavonoids in the aerial parts of *P. hydropiper* and *P. aviculare* have been developed.

2. The qualitative and quantitative composition of the main groups of biologically active substances (BAS) was detected.

3. The optimal technological parameters for obtaining dry crude extracts have been developed and established.

4. For the first time, laboratory regulations and the economic efficiency of the production of the summary phytopreparations from *P. hydropiper*;

5. Optimal ways of the separation and isolation individual compounds from crude extract have been developed and compiled for the standardization of substances. Twenty substances were isolated, of which: 10 flavonoids, 2 fatty acids, 3 sterols, 1 phenol carboxylic acid, 3 sesquiterpenes, 1 fatty acid ester. Arachidonic acid and isopolypiperic acid were isolated for the first time from the aerial part of *P. hydropiper.*

6. For the first time, the antimalarial, cannabinoid and opioid bioactivities of isolated compounds and fractions from crude extracts were studied for their possible use in medicine.

7. For the first time, the cannabinoid activity of 3,3'-dimethyl ellagic acid has been studied.

Theoretical significance of the work. The using of modern physico-chemical analytical methods is allowed us to obtain new information about the chemical compositions of some pharmacopoeial plants of the genus *Polygonum* L. and identified their quantitative amount. The developed methods of qualitative and quantitative determination of the amount of flavonoids in raw materials can be used to identify and assess the quality of plant raw materials. The results of the study of cannabinoid and opioid activitues can be used in further pharmacological studies to expand the field of using herb *P. hydropiper* and its preparations.

Practical significance of the study. The practical significance of the research is related to the use of local flora as the main raw material for the local pharmaceutical industry, as well as expanding the range of medicinal products of our own production from pharmacopoeial raw materials, which in the future will ensure the introduction of drugs into medicine. The practical value is determined by the results of bioscreening. If the raw materials are initially pharmacopoeial, the way for the introduction of substances from such raw materials will be shorter.

Main results:

1. Results of qualitative group and quantitative analysis of the main groups of biologically active substances, as well as the elemental composition of ash plants of the genus *Polygonum* L. and their phytopreparations. A qualitative analysis of three plants showed the group composition of the studied plants of the genus *Polygonum* L. is similar, but a quantitative assessment showed a divergence in the results on the content of the main groups of biologically active substances. Polysaccharides dominate in the aerial part of *P. aviculare* (5.13%), tannins - in *P. hydropiper* (2.11%), flavonoids - *P. aviculare* and *P. minus* (3.41% and 6.21%, respectively), amino acids - *P. hydropiper* and *P. minus* (7.72% and 6.16%, respectively) by results of quantitative content. The plant raw materials of *Polygonum* L. also contain a wide range of essential elements (trace elements) such as Zn,

Ni, Cr, Cu, Mn, Co, Mg in the ash residues after burning plant material (total ash *P. hydropiper* - 12.68%, *P. aviculare* - 8.21%, *P. minus* - 10.32%). A high content of phosphorus and manganese is consistent with their important role in the process of biosynthesis of products of primary and secondary metabolism.

2. Methods of spectrometric methods for the determination of flavonoids were standardized. A solution of aluminum chloride was used as a diagnostic additive. The maximum absorption of the complexes of flavonoids of *P. hydropiper* is observed at λ_{max} = 425 nm, and *P. aviculare* - at λ_{max} = 385 nm.

3. The influence of the nature of the extractant, the hydromodule, the method and duration of extraction on the yield of extractives compounds (EC) and flavonoids were studied.

In accordance with the results of the research, 50% ethyl alcohol was used as an extractant for *P. hydropiper* and *P. minus*, 70% ethyl alcohol for *P. aviculare*. Extracts were prepared in a ratio of 1: 7 - *P. hydropiper* and *P. minus*, 1:10 - *P. aviculare*. The degree of grinding of raw materials 3 mm - *P. hydropiper and P. minus*, 1 mm - *P. aviculare*. The optimal extraction method for obtaining dry biologically active extracts from *P. hydropiper* and *P. minus* was the bismaceration method; for *P. aviculare* - percolation. For *P. hydropiper*, the establishment of dynamic equilibrium concentrations in the solid-liquid system occurs after 24 hours (EC 23.77%, flavonodes 2.85%), for *P. aviculare* - after 12 hours (EC 23.27%, flavonodes 3.29%), for *P. minus* - after 24 hours (EC 22.84%, flavonoids 3.35%) for the first extraction and after 6 hours for three plants - for the second extraction.

4. For the first time, optimized technological flowcharts and material balances of the production of phytopreparations from three pharmacopoeial plants (*P. hydropiper, P. aviculare, P. minus*) were compiled. The yield of phytopreparations according to the proposed technologies for *P. hydropiper* was 25.25%, for *P. aviculare* - 23.95%, for *P. minus* - 24.73%. The economic rationale for the industrial production of dry extract from the *P. hydropiper* was calculated. Thus, the price of 100 grams of final product will cost more than 1,500 tenge.

5. The lipophilic fractions of dry extracts of two plants (*P. hydropiper* and *P. aviculare*) were studied by the chromato-mass spectroscopic method. Thus, 29 lipophilic substances were identified from the fraction of *P. hydropiper* extracted from the extract of *P. hydropiper* by retention time. Isobornyl thiocyanoacetate (6.85%), 5,5-dimethyl-4 - [(1e) -3-methyl-1,3-butadienyl] -1-oxaspiro [2.5] octane (11.87%), 6,6,9a- trimethyl-4,5,5a, 6,7,8,9,9a-octahydronaphtho [1,2-C] furan-1 (3H) -one (17.52%), phthalic acid, bis (2-ethylhexyl) ether (25.94%) were found in the highest concentrations. The *P. aviculare* RA-2-7 fraction contained 22 lipophilic substances, of which heptadecane (7.06%), octadecane (16.67%), tetracosan (12.75%) in the highest concentrations, calculated on the weight of the fraction.

6. The fatty acid compositions of the plants (*P. hydropiper, P. aviculare, P. minus*) were studied by gas-liquid chromatography. As a result of the analysis, 7 fatty acids in *P. hydropiper* and *P. minus* compounds were identified, 5 fatty acids in *P. aviculare*. In the

compositions of *P. hydropiper* and *P. aviculare*, caprylic acid 8: 0 predominates at concentrations of 63.37% and 40.43%, respectively; oleic - in *P. minus*. In smaller concentrations, *P. hydropiper* contains palmitic 16: 0 (13.39%) and linoleic 18:2∆6 (7.48%) acids; in *P. aviculare*, linolenic 18:3∆3 (20.40%) and palmitic 16:0 (16.40%) acids; in *P. minus*, linoleic acid 18:2∆6 (52.20%) and palmitic 16:0 (12.30%).

7. Chemical structures of 20 compounds have been isolated and established with using various variants of chromatography and data of chemical, physicochemical methods of analysis: stearyl sterate, isopolypiperic acid, 3, 3'-di-O-methylellagic acid, quercetin, taxifolin, β-sitosterol, β- sitosterol-β-D-glucoside (daucosterol), fugein, dendocarbin L, quercitrin, isoquercitrin, quercetin 3-O-methyl ester, isorhamnetine, tamarixetin, myrsetin, linoleic and stearic acids. Arachidonic acid and isopolypiperic acid were isolated for the first time from the aerial part of *P. hydropiper*.

8. The antimicrobial, antimalarial, opioid and cannabinoid activities of the isolated substances and fractions from dry extracts were studied. 7 extracts and 9 individual compounds were subjected to bioscreening studies. Moreover, opioid, and cannabinoid activities were studied for the first time for the species studied. 3,3’-di-O-methyl-ellagic acid and ethanol extract showed good modeling activity against CB1 receptors with 62.1 and 56.7% of binding, respectively. The ethyl acetate fraction showed good binding activity with the δ-subtype of opioid receptors (52.8%).

Estimation of completeness for the tasks solution. The tasks have been solved completely, since the quality indicators of the studied plant species (*P. hydropiper, P. aviculare, P. minus*) were studied, the qualitative and quantitative composition of the main groups of biologically active substances (BAS) in the studied plant species was established, the optimal technological parameters for obtaining dry extracts from the studied plants, a laboratory regulation has been drawn up for extracting total phytopreparations, optimal ways of the separation of their complexes of biologically active substances into individual compounds have been developed and selected substances have been identified, to study new types of activity of selected substances and fractions from dry extracts for further using in medicine.

Development of recommendations and baseline data on the specific use of the results. The obtained dry herbal phytopreparations can be used as medicinal substances, in particular in the treatment of patients with drug dependence.

Approbation of practical results. The main results of the thesis were reported and discussed at international conferences and forums: Young Generation Forum, World Conference of Scientists and Engineers (WCSE) (South Korea, Seoul, 2016), II Eurasian Forum of Young Scientists “YES-Forum” at the Foundation of the First President Of the Republic of Kazakhstan - Leader of the Nation (Almaty, 2016), VI International Scientific and Practical Conference "Medicinal plant growing: from past experience to modern technologies" (Poltava, 2017), XI International Scientific Conference "Innovative development and relevance of science in modern Kazakhstan ”(Almaty, 2017).

The personal contribution consists in choosing the direction of research, setting goals and objectives, experimenting, statistical processing of results and analysis of the

data obtained. The contribution of the author is crucial at all stages of the study. The author was directly involved in the collection and processing of literature data, the implementation of the experimental part, the analysis of the obtained results, their discussion, interpretation and conclusions, and the working out of the production technology of 3 total herbal remedies of broad activity developed in this work.

Relationship of the topic with the plan of scientific research and various state programs. The work was carried out within the framework of the Scientific and Technical Program of the Ministry of Education and Science of the Republic of Kazakhstan 2048 / GF4 "Development of universal cost-effective methods for the selective isolation of industrially significant classes of biologically active substances from plants of Kazakhstan" (state registration number 0115PK00563), deadlines 2015-2017.

Publications. According to the results of the dissertation, 9 scientific papers were published, of which 3 articles in editions from the list approved by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan: 1 publication in the rating journal “Chemistry of natural compounds” and 5 theses in materials of relevant international conferences.

Structure and scope of the dissertation. The thesis is presented on 148 pages of typewritten text and includes an introduction, 3 sections, a conclusion, 58 tables, 24 figures, a list of references from 262 titles and 4 appendix.