ANNOTATION

of the dissertation work by on the theme: **«Pharmacognostic study and prospects of application in medicine of *Nitraria schoberi* L., growing on the territory of Central Kazakhstan»** presented for the degree of Doctor of Philosophy (PhD) majoring in 6D110400 - Pharmacy

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Relevance of the Theme. According to the Government Resolution No. 132p as of October 6, 2020, the Program *Comprehensive Plan for the Development of the Pharmaceutical and Medical Industry for 2020-2025* was introduced in Kazakhstan. An important objective of this Program is to increase exports of pharmaceutical products in Kazakhstan, search for new pharmacologically active substances and their sources, as well as the creation of original, innovative, highly effective and harmless medicines.

The priority direction of the state policy in the field of health care in Kazakhstan is to increase the share of domestically produced medicines, replenishing the arsenal of medicines with plant preparations.

The extensive raw material base of Kazakhstan enables to create and implement original, competitive, economically accessible medicines based on local plant products.

A striking representative of the ancient desert flora is the plant *Nitraria schoberi*; it belongs to the extremely ornamental, edible, medicinal, as well as a powerful ameliorative object used in agriculture.

Nitraria schoberi L. is a member of the family *Nitrariaceae* Lindl. that is a valuable medicinal plant, widely used in folk medicine, both in our country and abroad, as a remedy with a unique combination of the main and associated mechanisms of pharmacological action. The plant is a prospective renewable raw material for the development and production of original medicines, has a sufficient exploitable stock in nature.

Despite the great interest of scientists from different countries of the world to the plant *Nitraria Schoberi L.*, the chemical composition and dynamics of accumulation of biologically active substances, the possibility of use in medical practice of this plant, growing in Central Kazakhstan, remains unstudied.

Due to all of the above, pharmacognostic study and the possibility of introducing into medical practice, raw materials *Nitraria schoberi L*., growing on the territory of Central Kazakhstan, is currently a relevant and priority issue in the field of pharmacy.

Purpose of the Work. Pharmacognostic study of the plant *Nitraria Schoberi L*., growing on the territory of Central Kazakhstan, determination of its component composition and pharmacological properties for use in medicine.

Objectives of the Study.

1. Morphological and anatomical study of the organs of the plant *Nitraria Schoberi L*., the determination of its diagnostic features, commodity quality

parameters of raw materials, microelement composition, stability index of active substances in raw materials and the determination of their shelf life.

2. To carry out standardization of raw materials *Nitraria Schoberi L*. and develop a quality specification. To prepare a product specification file for the plant product *Nitraria Schoberi L. fruits*.

3. To develop a technology for obtaining a substance from raw materials of *Nitraria Schoberi L.* and to standardize it. To prepare a product specification file and laboratory regulations for the substance obtained by ultrasound method from raw material of *Nitraria Schoberi L*.

4. To establish the substance of biological activity from the preparation *Nitraria schoberi* L. and determine the selection of promising extracts for the development of domestic medicines.

Objects of the study: above-ground and underground parts of *Nitraria schoberi L*. raw materials in the fruiting phase and their extracts obtained using the ultrasound method.

Subject of the study: determination of the main morphological and anatomical, diagnostic features of raw materials of *Nitraria schoberi L*., growing on the territory of Central Kazakhstan; study of the component composition for content of biologically active compounds in the organs of the plant *Nitraria schoberi L*. and in thick extracts; development of quality specification and product specification file for medicinal plant raw material fruits of *Nitraria schoberi L*. in accordance with the requirements of the Pharmacopoeia of Kazakhstan and the Pharmacopoeia of the EAEC; technology for the method of production of substances from the fruits of *Nitraria schoberi L*. by ultrasonic extraction and determination of optimal modes; development of quality specification and product specification file for the substance (thick extract) *Nitraria schoberi L*.; establishment of pharmacological activity of the substance from raw materials of *Nitraria schoberi L*: antimicrobial activity against strains of *S. aureus*, *E. coli*, antifungal against the yeast fungus *C. albicans*, antiaggregation, anticoagulation, anti-inflammatory, hepatoprotective, antioxidant and cytoprotective activity.

Method of study: The following methods of analysis were used in the work: macro- and microscopic analysis of raw materials on electron microscope "Biomed-4", histochemical analysis of raw materials, physical and chemical methods in accordance with the requirement of SPh RK, qualitative composition of natural compounds using methods of phytochemical analysis, extraction of plant raw materials using ultrasonic bath machine "Stegler" (China), component composition of substances and quantitative content of active substances in raw materials, substances using modern instrumental methods: high-performance liquid chromatography (HPLC/UV, HPLC/MS), UV spectrophotometry, radionuclides on atomic adsorption spectrophotometer, technological, pharmacopoeial, microbiological, pharmacological methods, corresponding to the requirements of regulatory documents applicable in the territory of the Republic of Kazakhstan.

Statistical processing of the obtained results in accordance with GPM 1.1.0013.15 *Statistical Processing of Chemical Experiment Results*.

Scientific novelty of the work.

Morphological and anatomical features of raw materials, its diagnostic features, qualitative and quantitative composition of biologically active substances, commodity analysis of the studied object growing in Central Kazakhstan were determined for the first time. Stability index was determined and shelf life was established. Instructions for collection, drying and storage of raw materials of *Nitraria Schoberi L* were developed.

For the first time an effective, economical, environmentally friendly technology of substance production from the fruits of *Nitraria schoberi L*. by ultrasound was developed and optimal modes were determined: dispersibility degree 3 mm, ultrasound radiation power 40 kHz, time 30 minutes, extraction process multiplicity 3 times, providing quantitative yield of pharmacologically active compounds.

For the first time using modern instrumental methods HPLC-UV and HPLC-MS, the component composition of the substance from the raw material of *Nitraria Schoberi* L. was determined, where flavonoids identified in the extract from the fruits of *Nitraria Schoberi L*. belong to the group of phenolic compounds. The dominant phenolic compounds are epicatechin (3.461 %), chlorogenic acid (1.489 %), gallic acid (0.984 %), p-coumaric acid (0.934 %), dihydroquercetin (0.273 %).

According to the data of bioscreening it was revealed for the first time that the substance of *Nitraria Schoberi L*. raw material shows a significant antibacterial activity against Eschericha coli, Staphylacocus aureuus, Bacillus subtilis, as well as causes growth inhibition of Candida albicans fungus culture.

For the first time it was revealed that the substance of the raw material of *Nitraria Schoberi L*. showed a significant hepatoprotective activity in the experimental study on animals (white rats weighing 230-390g) on the model of acute tetrachloromethane hepatitis.

For the first time it was revealed, as a result of the experiment on animals (15 white female and male rats weighing 210 - 440 g), that the substance from the raw material of *Nitraria Schoberi L*. at a dose of 50 mg/kg has anti-inflammatory activity on the model of acute exudative reaction. It was found that the thick extract of the studied species exhibits antiaggregation, anticoagulation, antioxidant activity comparable to the comparator drug.

Main statements presented for defense.

- Results of pharmacognostic, phytochemical studies, stability parameters of *Nitraria Schoberi* L.

- Technology of preparation of substance from raw material of *Nitraria Schoberi* L. using ultrasound method and experimental physicochemical, technological studies.

- Results of some pharmacological properties of the substance from the raw material of *Nitraria Schoberi* L.

- Product specification files for the substance from raw material of *Nitraria Schoberi* L. obtained by ultrasound using a solvent 70% ethanol.

Practical significance of the work.

As a result of morphological and anatomical study of *roots of Nitraria schoberi* growing on the territory of Central Kazakhstan, it was established that typical features *at the macroscopic level* are: *surface structure, color of bark* and inner part at the fracture, and diagnostic features *at the micro level* are: *structure of xylem vessels in tetrarch bundle, shape and color of periderm cells*.

Twelve phenolic compounds significant for the diagnosis of this object were identified by HPLC/UV and HPLC/MS in the extract from the fruits of *Nitraria schoberi* L. They belong to the groups of flavonoids (catechin, epicatechin), flavonols (rutin, quercetin, quercetin-3-glucoside, dihydroquercetin, phenolic compounds (rosmarinic acid, caffeic acid). It was found that the dominant phenolic compounds are epicatechin (3.461 %), chlorogenic acid (1.489 %), gallic acid (0.984 %), p-coumaric acid (0.934 %), dihydroquercetin (0.273 %), which contribute to the spectral characteristics of aqueous-alcoholic extracts from the fruits of *Nitraria schoberi L.*

- Methods of quantitative analysis for this type of plant product by differential spectrophotometry in terms of quercetin using a standard sample were developed.
- The following types of biological activities were first confirmed for the thick extract obtained by ultrasound from the fruits of *Nitraria schoberi* L.: hepatoprotective, antioxidant, antimicrobial, anti-inflammatory, antiaggregational, anticoagulation.
- Product specification file for medicinal plant product *Nitraria schoberi fruits* and its substance was developed.
- Laboratory regulations for obtaining the substance *Nitraria schoberi L. thick extract* were developed and approved.
- On the basis of the R&D Center of Non-Commercial Joint-Stock Company *KMU* the production of pilot batches of substances from raw materials of *Nitraria schoberi L.*, obtained by ultrasound for pharmacological studies was organized.

Publications.

The main provisions of the dissertation are reflected in the following 9 publications. 3 articles in the journals recommended by Committee for Quality Assurance in Science and High Education of the Republic of Kazakhstan, 1 article in an international scientific journal included in the database Scopus Q3, abstracts of 5 reports, including abstracts of 5 reports in the materials of international conferences, were published.

Conclusion:

For the first time according to the results of pharmacognostic study of medicinal plant product *Nitraria Schoberi* L. collected in populations of Karaganda region, morphological and anatomical and diagnostic features of raw materials were established: in appearance, shape, size of plant parts, also by the degree of lowering of leaf laminae, distinctive features of the structure of plant parts.

For the first time histochemical analysis of raw materials of *Nitraria Schoberi* L. was carried out for the presence of alkaloids, sesquiterpene lactones, flavonoids, phenolcarboxylic acids and polysaccharides.

Commodity parameters for fruits of *Nitraria Schoberi* L. were determined: loss in mass during drying within the limits (6,0 - 6,10)%; total ash is equal to (13,1-13,4)%; ash insoluble in hydrochloric acid is equal to the parameter (0,83-0,97)%; the sum of extractive substances was within the limits (33,2-33,8)%; extraneous organic impurities are not more than 0,02%, mineral impurities were absent in the studied object, the obtained results of the research are included in the product specification file.

The macro- and microelement composition and radionuclide content were determined in the raw material of *Nitraria sichoberi* L.

An environmentally friendly, economical, efficient technology for obtaining substances from raw materials of *Nitraria Schoberi* L. by ultrasound has been developed. The advantage of the developed technology is a 3-fold increase in the productivity of the technological process and a significant reduction in extraction time, increase in the yield of the sum of extractive substances.

Optimal parameters of extraction of raw material of *Nitraria Schoberi* L. using ultrasound method on ethanol of 70% concentration, is the quantitative yield of extractive substances, which is provided in the following modes of technological process: raw material/extractant ratio (1:20) at ultrasonic frequency of 40 kHz, extraction time of 30 minutes, at temperature of (20-25)°C.

Twelve phenolic compounds significant for the diagnosis of this object were identified by HPLC/UV and HPLC/MS in the extract from the fruits of *Nitraria schoberi.* They belong to the groups of flavonoids (catechin, epicatechin), flavonols (rutin, quercetin, quercetin-3-glucoside, dihydroquercetin, phenolic compounds (rosmarinic acid, caffeic acid). It was found that the dominant phenolic compounds are epicatechin (3.461 %), chlorogenic acid (1.489 %), gallic acid (0.984 %), p-coumaric acid (0.934 %), dihydroquercetin (0.273 %), which contribute to the spectral characteristics of aqueous-alcoholic extracts from the fruits of *Nitraria schoberi* L.

It has been established for the first time that the substance of raw material of *Nitraria Schoberi* L. obtained by ultrasound method shows significant antibacterial activity against strains of gram-negative bacteria *Eschericha coli*, gram-positive *Staphylacocus aureuus*, *Bacillus subtilis*, and also causes growth retardation of yeast fungus *Candida albicans*.

For the first time it was established that the substance of raw material of *Nitraria Schoberi* L. shows a significant hepatoprotective activity in an experimental study on animals on the model of acute tetrachloromethane hepatitis, "Carsil" was used as a comparator drug.

For the first time it was revealed, as a result of the conducted experiment on animals, the substance from the raw material of *Nitraria Schoberi* L. at a dose of 50 mg/kg has anti-inflammatory activity on the model of acute exudative reaction comparable to the comparator drug *Diclofenac sodium*.

The following types of biological activities were confirmed for the substance obtained by ultrasound from the fruits of *Nitraria schoberi* L. for the first time: antioxidant, antiaggregation, anticoagulation activities.

Product specification file for medicinal herbal raw material of *Nitraria schoberi fruits* was developed.

Product specification file for the substance (70% ethanol extract) of *Nitraria Schoberi* L. obtained by ultrasound method was developed.

Laboratory regulations for obtaining the substance *Nitraria schoberi* L. *thick extract* were developed and approved.

On the basis of R&D Center of Non-Commercial Joint-Stock Company *KMU* the production of pilot batches of substances from raw materials of *Nitraria schoberi* L., obtained by ultrasound for pharmacological studies was organized.