

ANNOTATION

Mykhailenko O.O. Pharmacognostic study of *Crocus sativus* and *Iris* genus plants: prospects for the complex use and optimization of growth conditions. – Qualified scientific work as the manuscript.

The thesis for the Doctor of Pharmaceutical Sciences Degree in Specialty 15.00.02 Pharmaceutical Chemistry and Pharmacognosy. – National University of Pharmacy, Ministry of Health of Ukraine, Kharkiv, 2024.

The dissertation is devoted to the theoretical justification and experimental solution of the scientific problem of ensuring a stable composition of bioactive components in herbal raw materials; determination of conditions and stages of proper cultivation and collection of Ukrainian medicinal plants using the example of *Crocus* and *Iris* genus plants for the creation of new herbal medicines with antiviral and anticancer effects based on them.

The planning of the experiment was done taking into account the "Quality by development" approach to ensure the optimization of the targeted search for anticancer and antiviral plant components from *Crocus sativus* and *Iris hungarica* herbal raw materials. The critical attributes of the plant extracts quality are defined, namely: raw materials quality control, auxiliary materials and the extraction process, taking into account the principles of "green extraction/chemistry". Using the Ishikawa diagram (qualitative risk assessment) and the Failure Mode and Consequence Analysis (quantitative assessment) approach, potentially critical production points affecting the plant extracts quality were identified, namely the cultivation and processing of herbal raw materials, the extraction process and the choice of solvents.

The selection of Q-markers for plant materials and extracts of *Crocus sativus* and *Iris hungarica* was substantiated using the Herbal chemical marker ranking system, pharmacognostic analysis and pharmacological screening of compounds and dry extracts. For *Crocus sativus*, the following markers have been suggested: crocin, rutin, isoquercitrin, ferulic acid, mangiferin, based on their use for the treatment of various cancer cell lines. Seven main marker compounds were selected for *Iris hungarica*: irisolidon, irigenin, tectoridine, kaempferol, quercetin, apigenin and mangiferin, taking

into account their anticancer and antiviral potential.

A comparative study of the chemical composition of 57 samples of *Crocus sativus* (saffron) from 13 countries of the world (including 35 samples from different regions of Ukraine) was conducted. The by-products of saffron production, such as leaves, perianth and corms were studied. The general chemical analysis of *Iris* genus plants included 38 species and 30 varieties from Ukraine, Azerbaijan, Lithuania and Latvia.

Standard operating procedures for the proper cultivation of medicinal plants have been developed for *Crocus sativus* and *Iris hungarica* in accordance with the WHO guidelines on Good agricultural and collection practices (GACP) for medicinal plants, the processing of herbal raw materials and the quality control for herbal raw materials for the pharmaceutical industry. In order to determine the optimal conditions for growing and/or collecting in the wild nature of *Crocus sativus* and *Iris* genus plants, a correlational study of the chemical composition of plants and their relationship with environmental factors (geographical, climatic and edaphic) was conducted.

For the first time, a comparative analysis of 35 saffron samples from 12 regions of Ukraine was conducted. Using the HPLC method, it was established that the concentration of crocin and picrocrocin is higher for raw materials from the northeastern regions of Ukraine and decreases closer to the southwestern regions of cultivation. It was established that the content of phenolic compounds and apocarotenoids (crocin t-4GG; crocin t-2G; crocin c-4GG; crocin c-3Gg) in *Crocus* stigmas is correlated with the duration of solar radiation, solar UV index and soil type. Crocins, picrocrocin, safranal and rutin can also serve as markers of saffron quality. The expediency of using the method of grinding herbal raw materials in liquid nitrogen during the sample preparation, which increases the yield of crocins by 3.5 times compared to the usual grinding. Analysis of the quality of Ukrainian saffron in accordance with ISO 3632 indicators showed that the majority of samples are classified as category I (higher quality).

The conducted research, as well as a comparative analysis of the monograph of the European Pharmacopoeia 9.0, the Deutscher Arzneimittel Codex and ISO 3632 on *Crocus sativus* stigmas, made it possible to develop a draft monograph "Saffron sativus stigmas^N" for introduction to the national part of State Pharmacopoeia of Ukraine 2.4.

HPLC and HPTLC methods are proposed for detection of possible adulteration of saffron. Crocin, which is a marker of saffron quality, as well as picrocrocin, were not detected in the tested traditional counterfeits (turmeric, safflower, calendula, marigolds, yellow tulip stamens). The HPTLC data were correlated with the HPLC analysis data, where the characteristic peaks of crocin (Rt 28.37 min, UV λ_{\max} 261, 440, 466 nm) were absent in the counterfeit extracts.

An HPLC technique for the determination of colchicine in *Crocus* raw materials is proposed to resolve the issue of possible falsification and toxicity of saffron. The analysis showed that the plant does not contain colchicine. The proposed method was successfully tested for *Colchicum autumnale* and showed a good separation and identification of colchicine and the correctness of the method for both positive and negative results.

For the first time, the Standard operating procedure for obtaining working standard samples of picrocrocin and crocins (*trans*-4GG, *trans*-2G, *trans*-3Gg) by preparative chromatography was developed. Compounds were isolated using a Symmetry Prep C18 column (300×19 mm×7 μ m). The purity of crocins and picrocrocin was more than 97%. The proposed method is simple and reproducible, economical for routine quality control analysis of saffron.

Environmental methods (cold plasma, vacuum, electromagnetic field) of stress treatment of *Crocus sativus* were applied to influence the kinetics of plant germination in field conditions, compounds content, extracts antioxidant effect. All stressors significantly (42-74%) increased the number of leaf trichomes, the total phenolics content and free radical activity in *Crocus* perianth and leaves. By HPLC method was identified and quantified the 26 compounds, including 23 crocetin ethers, as well as rutin, picrocrocin and safranal, in the saffron test samples. The electromagnetic field had the greatest impact on the biosynthesis of secondary metabolites of saffron: a 7- to 9-fold increase in the content of crocin derivatives was noted.

The study of the chemical composition of the by-products of saffron production, such as *Crocus* leaves, perianth and small, low-quality corms are showed a significant polyphenolic, amino acids, carboxylic acids and macro- and microelements in the raw materials. The chemical composition of eight types and varieties of *Crocus* leaves,

among which *Crocus speciosus* turned out to be the most promising, and five flower samples of different *Crocus* varieties were studied separately.

For the first time, 16 phenolic compounds (ferulic acid, chlorogenic acid, neochlorogenic acid, astragalín, rutin, quercetin, kaempferol, isorientin, isorhamnetin, isoquercitrin, tectoridine, nigricin, iristectorigenin B, mangiferin) were isolated from the ethyl acetate fraction of *Crocus sativus* perianth by column chromatography. Soforaflavonolósido and cosmosin were isolated for the first time. The structures of the compounds were determined by NMR spectroscopy.

An HPLC-DMD method for the analysis of phenolic compounds of *Iris* species was developed, which was tested on rhizomes of *Iris pallida*, *Iris hungarica*, *Iris sibirica*, *Iris variegata*. Chromatographic separation of components was carried out using a Shimadzu HPLC system equipped with an ACE C18 column (250 mm × 4.6 mm) with gradient elution with a 0.1% solution of acetic acid in water-acetonitrile (A) and acetonitrile (B) with increasing polarity from 5 to 95 %. In the future, the developed technique made it possible to identify and establish the content of 25 phenolic compounds in the studied samples. The structure of compounds was confirmed by the method of ultra-efficient liquid chromatography-mass spectrometry in the negative ion mode.

The study of the environmental factors influences on the phenolic compounds content in rhizomes and leaves of *Iris germanica*, *Iris pseudacorus*, *Iris sibirica*, *Iris halophila* from 17 populations of Ukraine, Lithuania and Latvia was conducted. The dominant compounds were mangiferin, tectoridin, germanaism B, 5,6-dihydroxy-7,8,3',5'-tetramethoxyisoflavone, iristectorigenin B, nigricin, irigenin. Correlation studies established a positive effect of the duration of solar radiation and a negative effect of the amount of annual precipitation on the quantitative content of phenolic compounds in *Iris* species rhizomes. A high content of phosphorus and potassium in the soil significantly positively affects the phenolic compounds amount, while the nitrogen content does not have a significant effect on the compounds accumulation.

The column chromatography method for the compounds isolation from *Iris hungarica* rhizomes has been improved. As a result, 30 phenolic compounds were isolated, including the rare isoflavonoids irisolidone, irigenin, irisolone, irilon,

kikkalidone, ononin, genistein, daidzein, formononetin, nigricin, germanaism B, and the xanthones mangiferin and isomangiferin. In addition, isoflavones tectorigenin, tectoridin, iristectorigenin B, iristectorin B, 5,6-dihydroxy-7,8,3',5'-tetramethoxyisoflavone and flavones hyperoside, apigenin, kaempferol, quercetin, isoquercitrin, embinin, cosmosinin; hydroxycinnamic acids: chlorogenic, neochlorogenic, caffeic, ferulic, cinnamic, coumaric were identified by comparison spectral data with those available in the literature. The compound structures were determined using 1D and 2D NMR spectroscopy.

Screening of the total γ -pyrones content in 18 samples of *Iris* species and varieties was carried out to substantiate the choice of mangiferin sources. The total content was from $0.25 \pm 0.08\%$ to $1.64 \pm 0.12\%$. *Iris hungarica* leaves had the highest content and were chosen for the development of a method for obtaining mangiferin. The proposed method is based on step-by-step purification of plant raw materials, extraction, filtration, drying and evaporation and provides a substance yield of 1.05%.

The carboxylic acids composition of *I. graminea*, *I. sibirica*, *I. halophila*, *I. pallida*, *I. variegata*, *I. pumila*, *I. musulmanica*, *I. carthaliniae*, *I. medwedewii*, *I. musulmanica* rhizomes and leaves has been studied. The content of saturated fatty acids in *Iris* species rhizomes reviled (from 15% to 53%), and the content of unsaturated acids was higher in the leaves. Citric, malic, and oxalic acids are dominant among organic acids. A study of terpenoids of 27 species and varieties of *Iris* genus plants was carried out. The total yield of essential oil of *Iris* rhizomes is from 0.02% to 0.58%. The dominant compound is α -iron (up to 2.85%). Norterpeneoids and their derivatives: β -damaskenone, β -ionone-5,6-epoxide, β -ionone were discovered for the first time in *Iris* species.

Determining the optimal harvesting terms of *Iris* rhizomes and leaves showed that the content of xanthones, phenolic acids, flavonoids and isoflavonoids depends on the growing season and has a maximum accumulation in summer. Xanthone-mangiferin is recognized as the dominant component of *Iris* species leaves.

The study of the qualitative composition and quantitative content of marker compounds in the raw materials of *Iris hybrida* *Tcikavinka*, *Iris hybrida* *Talibo*, *Iris hybrida* *Widcombe Fire* was carried out using monocomponent nutrition with iron chelate (Fe^{2+}), potassium chelate (K^+) and magnesium sulfate (Mg^{2+}) during the

growing season. The obtained data on the total polyphenolics content were directly correlated with the data on the quantitative content of individual compounds by the HPLC method. The content of the isoflavones genistin (up to 9.67 mg/g), irigenin (up to 3.52 mg/g) and irisolidone in the rhizomes, and chlorogenic acid and mangiferin in the leaves significantly increased.

Dry extracts (water and ethanolic, 70%) were obtained from *Crocus sativus*, *Iris hungarica*, *Iris sibirica*, *Iris variegata* herbal raw materials for pharmacological research. Their chemical composition was determined and the results of pharmacological screening were discussed, namely: *in vitro* studies of plant extracts and individual compounds, including neuraminidase inhibitory activity, antiviral (enterovirus D68, influenza virus H1N1, coronavirus 229E), anti-inflammatory, antioxidant, antiallergic, expression NRF2, lipid-forming, cytotoxic, antibacterial activities.

For *in silico* prediction, 18 irises phenolics were plotted using ChemGPS-NP to correlate the observed activity of selected compounds with clinical anti-influenza and anti-enteroviral drugs. Irisolidone, kikkalidone and kaempferol were the most active against enterovirus. Further *in vitro* studies confirmed the anti-enteroviral activity of D68: irisolidone (1.95% inhibition at 50 μ M, IC_{50} 9.2 μ M), kikkalidone (5.93%), kaempferol (14.83%). In addition, apigenin (100% inhibition at 50 μ M), kaempferol (92%), quercetin (48%) had a pronounced anti-influenza effect, which was confirmed by the analysis of neuraminidase inhibition. Extracts of *Iris hungarica* rhizomes also showed pronounced cytotoxic, anti-influenza, anti-enteroviral effects due to polyphenolics.

The water extract of saffron showed a pronounced cytotoxic effect on the glioblastoma line, and the ethanolic extract significantly reduced the viability of melanoma and triple-negative breast cancer. Dry extracts of *Crocus* corms showed a very significant anti-allergic effect (at the same level as dexamethasone). The ethanolic extract of *Crocus* corms showed a pronounced protective effect against the influenza virus of 30-40% protection, and also had a powerful cytotoxic effect against neuroblastoma cancer cells (EC_{50} 0.018 mg/mL) and against U-87 glioblastoma cells (EC_{50} 0.02 mg/mL). Extracts of *Crocus* leaves and perianth showed a pronounced antioxidant effect. All obtained extracts belong to the V class of toxicity according to K.K. Sydorova

classification. An *in vivo* study showed the presence of an anti-inflammatory effect of *Crocus* corms and *Iris hungarica* rhizomes extracts (at a dose of 200 mg/kg). Standardization parameters for raw materials and extracts of *Crocus* were developed.

Key words: Iridaceae family, *Crocus sativus*, *Iris hungaria*, *Iris variegata*, *Crocus* species, *Iris* species, *Iris* varieties, phytochemical composition, natural compounds, plant ecology, chromatographic studies, analytical methods, pharmacological studies.