PRELIMINARY CLASSICAL CHROMATOGRAPHIC ANALYSIS OF PHENOLIC COMPOUNDS OF *I. APHYLLA SUBSP. HUNGARICA*

[1]Mykhailenko O., [2]Kovalyov V., [3]Buidin Y., [3]Chetvernya S., [4]Orlova T., [1]Georgiyants V.

[1] Department of Pharmaceutical Chemistry, National University of Pharmacy, Kharkiv, Ukraine

[2] Department of Pharmacognosy National University of Pharmacy, Kharkiv, Ukraine

[3] M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

[4] Department of the Floriculture, Botanical Garden of the Kharkiv National University named after V.N. Karazin, Kharkiv, Ukraine

Mykhailenko.farm@gmail.com

Iris spp. (*Iridaceae*) are distributed in European countries, including Italy, France, Spain, and Ukraine [1]. Its rhizomes are called orris root and have been used as a perfume ingredient as well as herbal raw material are used in traditional medicines. Although a few norisoprenoids including *α*- and *β*-irones, squalene, different carboxylic acids and amino acids have been identified in *I. hungarica*, there has been no systematic phytochemical study of its secondary metabolites. The present investigation of the chemical components of the rhizomes of *I. aphylla* subsp. *hungarica* resulted in the isolation of new isoflavonoid glycosides (irisolidone, kikkalidone, irigenin, irisolone, irilone, genistein, daidzein, ononin, formononetin), as well as xanthone mangiferin for this species. This abstract deals with the preliminary structural elucidation of the isolated compounds, based on spectroscopic analysis.

Material and methods. Rhizomes of *I. hungarica* Walds. et Kit. (*Iridaceae*) were collected in Kharkiv Botanical Garden of the Kharkiv National University named after V.M. Karazin in May 2017. The raw materials were dried at ambient temperature at 20–24 °C and used for the chemical analysis. The rhizomes of *I. hungarica* were extracted with 70% ethanol. After removal of the solvent, the concentrated ethanolic extract was prosessed up successively with chloroform, ethyl acetate, and butanol. The EtOAc and $CHCl_3$ extracts combined and subjected to column chromatography on silica gel. Compounds were identified based on their physical and spectroscopic data.

Results and discussion. *Chemical analysis on functional groups*. The qualitative analysis of functional groups showed that the following groups are present in the isolated compounds. The compounds **3, 6, 7, 9** gave a black-green color with 1% alcohol solution of $FeCl_3$ and purple-brown color with staining with 1% aqueous solution of $FeCl_3$. The compounds **2** and **5** gave a green color when treated with 1% alcohol solution of $FeCl_3$ and a light brown color with 1% aqueous solution of $FeCl_3$. This indicated the presence of phenolic hydroxyl groups in **2** and **5**. Also, a violet color of compounds was observed with chromotropic acid in conc. H_2SO_4. This indicated the presence of methoxyl moiety in the molecules of **2-4**, **10**. Cyanidin test for the flavonoids determination was performed and the compounds were colored bright yellow upon the reaction with metallic magnesium powder and conc. HCl. The presence of the methylenedioxy group was established by qualitative reaction with 5%

solution of gallic acid in the presence of concentrated sulfuric acid and chromotropic acid (Labat test) [2]. Presence of a green color compared with a blank test indicated the presence of methylenedioxy group in **4** and **6**.

Spectral data. The UV spectra of isolated compounds **2-7, 9-10** in ethanol showed only one absorption peak between 250-276 nm and "shoulder" at 300-340 nm suggesting the isoflavone skeleton. This is in accordance with the hydroxyl pattern of the B-ring of compounds (4′-OH). The UV spectrum of compound **1** (irisolidone) showed at 268 and 330 nm (sh). Addition of anhydrous $AlCl_3$ to the solution shifted the λ max to 278 nm with no change after the addition of HCl. Further addition of NaOAc showed a bathochromic shift of 8 nm of this band. The UV spectrum data supposed that the molecule contains an isoflavone skeleton together with a chelated 5-OH group and a 7-OH group. Compound **4** (irilone) was isolated as yellow crystalline powder. The UV spectrum gave band II and band I at 270, 330 nm (sh.) respectively, indicating the presence of an isoflavone skeleton. The low intensity peak (Band I) was associated-with absorption due to B-ring phenyl system and intense peak at 269 nm (Band II) involved the A-ring benzoyl system. Addition of $AlCl_3$ showed a bathochromic shift of Band II by 13 nm, which did not change after the addition of HCl. This phenomenon indicated the presence of a 5-OH group in the molecule. The UV spectrum was unaffected by the addition of fused NaOAc to the system. Compound **5** gave positive reaction result in the test on phenolic hydroxyl groups, showing blackish-green color with alcoholic 1% $FeCl_3$ and violet-brown color with aq. 1% $FeCl_3$ solutions. The UV spectrum of **5** showed the λ max absorptions at 268 and 330 nm (sh), suggesting the isoflavone skeleton. In addition, the proton resonance for isoflavone C-2 was located at δ 8.45 (1H, s) ppm, which also confirmed the isoflavone nature of the ring. Acid hydrolysis of **5** with 10% H_2SO_4 gave aglycone **1** and *D*-glucose which were identified by co-PC and co-TLC. After removal of several isoflavonoids, the column was eluted with a mixture of chloroform-ethanol (85:15) to afford a compound **11**. It was recrystallized from EtOH to give pale yellow amorphous powder with a molecular weight of 422.35 g/mol. The melting point (anhydrous) of **11** has been reported to be 271˚C. Compound **11** is soluble in solvents such as DMSO, water and methanol. Compound **11** gave a positive reaction (greenish color) in the test on the phenolic hydroxyl groups using 3% $FeCl_3$. The UV spectrum of **11** showed the λ max absorptions at 369, 318, 259 and 241 nm suggesting the xanthone skeleton. Characteristic bathochromic shift of UV absorption maxima of **11** upon addition of anhydrous NaOAc indicated the presence of free hydroxyl group at C-3. Further addition of 3% solution of boric acid led to a characteristic bathochromic shift, which is typical for two OH-groups of ring B at *ortho* position.

References

1. Кречун А.В., Михайленко О.О., Ковальов В.С., Орлова Т.Г. Актуальні питання фармацевтичної і медичної науки та практики 12, (2019) 135-140;
2. Markham K.R. Techniques of Flavonoid Identification. Academic Press, London. 1982.
3. Andersen O.M., Markham K.R. Flavonoids: chemistry, biochemistry and applications. 1st Ed. CRC Press; 2005. 1256 p.