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CAROTENOIDS OF LENTILS LIPOPHILIC EXTRACT

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Introduction. Lipophilic extracts of plants contain the most important classes of biologically active compounds, such as lipids, carotenoids, tocopherols, chlorophylls, sterols and fatty acids. They have different types of biological action depending on the composition and structure of the individual components.

Among the known plant pigments, carotenoids are the most common and differ in structural diversity and a wide range of biological effect. In higher plants, carotenoids are synthesized and localized in cell plastids, in which they are connected into photosensitive complexes, participating in the process of photosynthesis and protecting plants from oxidative stress caused by excessive lighting. In plants, they are secondary metabolites and are divided into two groups: oxidized xanthophylls, such as lutein, zeaxanthin, violaxanthin and carotenoid hydrocarbons, such as β- and α-carotenes and lycopene [1].

Carotenoids are considered to be one of the most powerful traps of singlet oxygen. It is the antioxidant properties of these compounds that largely determine their biological activity.

For example, carotenoids normalize the processes of regeneration, disorders in the wound healing process. Many medicaments have been developed on their basis and are being used in medical practice [1, 3].

Lentils also contain compounds of a lipophilic nature that is why we decided to obtain and investigate a lipophilic extract from the nutritive lentil grass for a complex research, and later for the use of medicinal raw materials.

Purpose of the study. Lentil (*Lens culinaris*) is cultivated plant of the legume family, which also contains lipophilic nature compounds. Therefore, we decided to investigate carotenoids of lentils herb for a comprehensive study and further usage of raw material in medical practice.

Materials and methods. To study carotenoids the chloroform fraction was obtained from the lentils herb. Lipophilic fractions have been obtained from the investigated raw material for the present lipophilic effects. Air-dry, ground grass (2800) was exhaustively extracted with chloroform in a Soxhlet apparatus. The chloroform extract has been evaporated to complete removal of the extractant. The percentage content of the amount of lipophilic substances in the raw material was determined by the gravimetric method.

The content of lipophilic fraction in the grass of nutritive lentil is 3,90 ± 0,21%. Separation of lipophilic extract components were achieved by TLC on “Sorbfil” plates in such solvent systems: hexane-acetone (6: 4) - I direction and hexane-acetone (6:2) – II direction. Quantitative determination of carotenoids in the lipophilic fraction was carried out spectrophotometrically on a SF-46 spectrophotometer.

To study the fluorescent components of the obtained lipophilic fractions, three-dimensional fluorescence spectra were obtained by three-dimensional scanning spectrofluorimetry in ultraviolet and visible spectral ranges using a Hitachi F4010

spectrofluorimeter with the assistance of PhD in Chemical Sciences O. Roshal. Spectrum measurements were performed in the excitation and emission ranges from 220 to 800 nm in 10 nm increments. Further processing of records with the construction of three-dimensional graphs was used with the help of the software package Spectra Data Lad, developed at the Research Institute of Chemistry of KhNU named after Karazin.

To record the fluorescence spectrum, 20 mg of lipophilic extract was dissolved in 5 ml of acetone. The resulting solution was placed in a cell of a Hitachi F4010 spectrofluorimeter and in the mode of scanning at change of the wave with the increments of 10 nm in the ultraviolet and visible range of 220-800 nm the fluorescence spectrum was recorded. Further, the processing of fluorescence spectra with the construction of three-dimensional graphs was performed using a computer.

The results of the study and their discussion. The localization of carotenoids on chromatograms was determined by characteristic yellow or orange coloration in visible light, and brown fluorescence in UV light. To clarify the results of visual control the chromatograms were sprayed with the 2% solution of n-dimethylaminobenzaldehyde in a mixture of ethanol and hydrochloric acid. After the treatment they were dried at 80-90° C for 5-7 minutes. The spots corresponding to carotenoids were dyed pink-violet. Carotenoids were also visualized by spraying plates with the 10% alcohol solution of phosphomolybdic acid and subsequent heating them in a drying oven at 60-80° C for 5 minutes. Blue spots of carotenoids were observed on a yellow-green background [5].

The analysis of three-dimensional fluorescence spectra of the lipophilic extract that has been carried out, as well as the analysis of the projected fluorescence spectrum on the expansion / radiation plane, presented in logarithmic scales of intensive development, facilitated a more detailed identification of the qualitative content of the investigated raw material. These spectra have a characteristic appearance for the corresponding materials and are clear identification "prints" that contain not only the complex composition, but also allows you to justify the quantitative content of the component.

Thus, in the investigated lipophilic extracts there is a series of peaks in the excitation regions. Peaks in the excitation regions of λexc 270-330 nm and the emission of λem 350-400 nm correspond to the emission of simple phenolic compounds and indicate the presence of carotenoids. Substances which on the three-dimensional spectrum give excitation peaks λexc 250-300, 330-400 nm, and the emission peak λem 450-500 nm were attributed by us to derivatives of flavones and flavonols.

Quantitative determination of carotenoids in the lipophilic fraction: 0,02 g of a lipophilic extract were placed in a 25 ml volumetric flask, were dissolved in 96% ethanol until complete dissolution and the volume of the volumetric flask was adjusted to the mark with the same solvent (solution A). Then 2 ml of Solution A were diluted to 10 ml with 96% ethanol. The content of the individual pigments was determined using a three-wave method, the optical density of the resulting solution was measured by SF-46 spectrophotometer at 440 nm in a cuvette with 10 mm layer thickness [2].

The concentration of carotenoids in the resulting solution (C, mg/ l) was calculated by the Wettstein equation [2,4]. For calculation the content of carotenoids sum (X, %) in the lipophilic fraction such formula was used:

$$X = \frac{C \cdot 25}{m \cdot 2 \cdot 1000},$$

where C - concentration of carotenoids in solution, mg / l;

m - weight of lipophilic extract sample, g.

Conclusions. As a result, at least 4 substances of a carotenoid nature were detected in the lipophilic extract. The content of carotenoids in the investigated lipophilic extract was 0.35 ± 0.08%.

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