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IN-VITRO EVALUATION OF THE ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *VACCINIUM VITIS-IDAEAE* L. LEAVES

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Biologically active substances in lingonberry leaves (*Vaccinium vitis-idaea* L.) have antioxidant properties. This study selected the optimal extraction method to obtain extracts with a high content of biologically active components with antioxidant activity from the leaves of *Vaccinium vitis-idaea* growing in Yakutia. The data obtained allow us to conclude that biologically active substances isolated by alcoholic extraction from the leaves of *Vaccinium vitis-idaea* can be used in medicine to find approaches to regulating pro-oxidant processes in the human body under various pathological conditions.

Keywords: lingonberry, biologically active substances, gravimetric method, model system.

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Introduction. For the treatment and prevention of new viral diseases, there is a need to search for new drugs. Plant-origin preparations with a high content of biologically active substances (BAS) are highly interesting due to their safety, availability, and renewal of biological resources. The quantitative and qualitative content of BAS of plant raw materials depends on natural conditions [6]. For example, the accumulation of BAS by plants can be influenced by the following factors: growing region conditions, cultivation method, weather conditions, maturation stages, soil, and extraction method [4, 5]. Thus, the harsh and unique abiotic factors of the plant growing environment in Yakutia contribute to the active accumulation of biologically active compounds in plants during a relatively short growing season.

In pharmaceutical practice, the most interesting are medicinal plants con-

taining phenolic compounds with pronounced antioxidant properties.

Common lingonberry (*Vaccinium vitis-idaea* L.) is a perennial shrub belonging to the Heather family (Ericaceae). *Vaccinium vitis-idaea* grows in coniferous and mixed forests, in the mountain and plain tundras, and sometimes on peat bogs. The entire aboveground part of *Vaccinium vitis-idaea* is used as a medicinal raw material: shoots, leaves, and berries. In 2018, common chasteberry was included in the State Pharmacopoeia of the Russian Federation (XIV edition). In medicine, the leaves of *Vaccinium vitis-idaea* are used as a diuretic, antimicrobial, and anti-inflammatory agent.

According to literature data, components of *Vaccinium vitis-idaea* exhibit anti-inflammatory, neuroprotective, hypoglycemic, antioxidant, and antitumor properties, which are determined by the

presence of biologically active substances of different natures [7, 8, 9]. *Vaccinium vitis-idaea* leaves have been shown to contain phenolic glycosides such as arbutin, methylarbutin, vaccinin, hydroquinone, organic acids, including gallic, tartaric, ellagic, cinnamic, ursolic acids and flavonoids represented by quercetin, quercitrin, isoquercitrin and quercetin-3-arabinoside, including tannins and catechins [11].

The study aims to find an optimal extraction method for obtaining extracts with a high content of biologically active components with antioxidant activity from the leaves of *Vaccinium vitis-idaea* growing in Yakutia.

Material and methods. The work was carried out in the laboratory of pre-cancerogenesis and malignant tumors of the Department of Epidemiology of chronic non-infectious Diseases, Yakut Science Centre of Complex Medical Problems, within the framework of the research work "Regional features of biochemical, immunological and morphological parameters in the indigenous and native population of the Republic of Sakha (Yakutia) in norm and pathology."

Plant raw materials and preparation of extracts. Ethyl and aqueous extracts of *Vaccinium vitis-idaea* leaves were used in this study. Plant raw materials were collected during the fruiting phase (August-September) in Namsky District of the Republic of Sakha (Yakutia). The leaves were dried naturally at room temperature without access to direct sunlight. After the drying stage, the plant material was subjected to grinding on a vibrating ball mill "GT-200" ("GRINDER"). Extraction was carried out by single maceration for 60 minutes at a temperature of 60 °C. Distilled water and ethyl alcohol in different concentrations from 20 to 95%, in increments of 10%, were used for extraction. By weight, the ratio of dried raw material of *Vaccinium vitis-idaea* leaves and water/alcohol extractant was 1:10. The obtained extracts were centrifuged, then the soluble fraction of the extract was passed through a filter with a pore diameter of 0.22 µm ("Membrane Solutions").

Determination of extractive substances content. For general quantitative analysis of extractive substances and identification of conditions that have the maximum extraction, the amount of biologically active components and ballast substances were determined depending on the type of extractant used.

Quantitative determination of the content of extractive substances was carried out gravimetrically, according to the meth-

od described in the state pharmacopeia "1.5.3.000615", using lyophilization. After extraction, ethyl, and aqueous extracts were transferred to accurately weighed containers for drying. Lyophilization of extractables was carried out on a Free-Zone (Labconco) lyophilic dryer (150Pa, -52 °C) until complete sublimation of the solvent. The weight of the extract before and after lyophilization was measured to determine the total mass of the extractable.

The content of extractive substances was calculated according to the formula specified in the method "Determination of extractive substances content in medicinal plant raw materials and medicinal plant preparations."

Determination of the antioxidant activity of aqueous and alcoholic extracts. To evaluate the antioxidant activity (AOA) of the studied extracts, we used the standard technique of lipid peroxidation (LPO) on the model system of yolk lipoproteins (YLP) [2]. Oxidation of unsaturated fatty acids was initiated by adding iron (II) sulfoxide. Optical density was measured at a wavelength of 530 nm on a Cary 100 UV-Vis spectrophotometer (Agilent Technologies). Antioxidant activity (X) was calculated in percent by the formula:

$$X = \frac{E_k - E_o}{E_k} \times 100,$$

Ek - optical density index in the control sample without *Vaccinium vitis-idaea* leaf extract; Eo - optical density index in the experimental sample.

In this study, statistical processing of the obtained data was done using the software "SPSS Statistics" (version 27.0.1). The normality of the distribution of the data set was checked using the Kolmogorov criterion. Data sets with normal distribution were compared using

Student's t-criterion, and data differing from normal distribution were compared using the Mann-Whitney statistical U-criterion. The significance level for accepting the null hypothesis was accepted at $p < 0.05$.

Results and Discussion. In a study by Bujor et al. (2018) using a method to evaluate antioxidant activity by the ability of the studied objects to reduce the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), it was shown that the antioxidant properties of BAS of alcoholic extracts isolated from the leaves are higher than those from the fruits of *Vaccinium vitis-idaea* [10]. This study suggests that the leaves of *Vaccinium vitis-idaea* are a valuable biological raw material with a unique qualitative and quantitative composition of BAS. In this regard, we studied the antioxidant properties of aqueous and alcoholic extracts of *Vaccinium vitis-idaea* leaves.

Determination of the content of extractable substances after filtration, through a membrane with a pore size of 0.22 µm, of aqueous and alcoholic extracts of *Vaccinium vitis-idaea* leaves showed that with increasing concentration of ethyl alcohol in the extractant, the yield of the total content of active and ballast substances decreases. According to literature data, the leaves of *Vaccinium vitis-idaea* of Central Yakutia contain up to 9.5% arbutin, up to 35% tannins, and ascorbic acid - 27% [1]. The study's results to determine the content of extractable substances confirm that the extractable substances of *Vaccinium vitis-idaea* leaves are mainly represented by water-soluble compounds: simple phenols, carbohydrates, tannins, and organic acids.

The antioxidant activity of *Vaccinium vitis-idaea* leaf extracts was investigated on a model system of yolk lipoproteins. The model system has a constant lipid

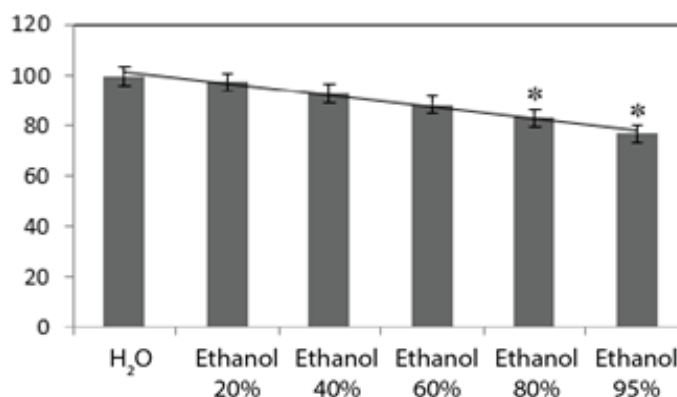


Fig. 1. The content of extractive substances in aqueous and ethyl extracts of *V. vitis-idaea* leaves. The ordinate axis shows the content of extractive substances expressed in percent. The abscissa axis shows the type of extractant used; * - $p < 0.05$.

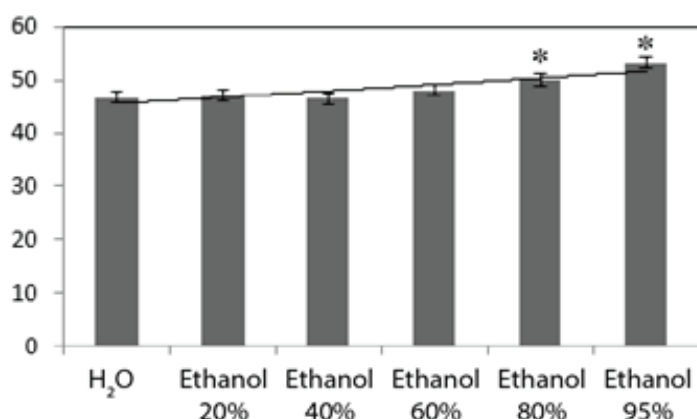


Fig. 2. Total antioxidant activity of aqueous and ethyl extracts of *V. vitis-idaea* leaves in the model system of yolk lipoproteins. The ordinate axis shows antioxidant activity expressed in percent. The abscissa axis shows the type of extractant used; * - $p < 0.05$.

composition. It contains two lipid-protein complexes, corresponding to the lipid and protein composition of very low and low-density human blood plasma [2]. When diluted in phosphate buffer, egg yolk phospholipids form bilayer micelles - liposomes. In this system, oxidation occurs on the surface of liposomes carrying a charge on the polar heads of phospholipids. The acceleration of free-radical oxidation processes accompanies the introduction of iron (II) sulfoxide into the system, while spontaneous oxidation in this model is extremely insignificant. The introduction of antioxidants into the model system inhibits the intensity of peroxidation processes. Consequently, the model system of yolk lipoproteins can determine the studied objects' antioxidant properties (plant extracts, biological fluids).

Our study's results showed that the extracts' antioxidant properties tend to increase depending on the increase in ethyl alcohol concentration. It was observed that the increase in antioxidant properties of the extracts starts from 60% alcohol extract and further.

Alcoholic extraction of *Vaccinium vitis-idaea* leaves allows more intensive extraction of active substances (phenols, flavonoids, and catechins) from the ob-

ject of study. Interesting are the works conducted by Raudone et al., where the authors showed that alcoholic extracts of *Vaccinium vitis-idaea* leaves are characterized by a high content of phenolic substances, and the antioxidant activity of the extracts depends on their composition [3]. Preliminary data allow us to conclude that unique biologically active components isolated by alcoholic extraction from *Vaccinium vitis-idaea* leaves can be used in medicine to find approaches to regulate pro-oxidant processes in the human body in various pathological conditions.

Conclusion. The obtained data indicate that the extraction of biologically active phenolic compounds from *Vaccinium vitis-idaea* leaves is more intensive in 95% ethyl alcohol compared to aqueous and other aqueous-alcohol solutions, as evidenced by the increase in the total antioxidant activity carried out on the model of yolk lipoproteins.

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