Investigation of antioxidant capacity of the extracts of bilberry (VACCINUM MYRTILLIS L.) by voltammetry

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Abstract. This paper deals with the urgent issue of the search of new drugs based on plant raw materials that have an influence on various stages of oxidation processes occurring in the human body. The aim of this paper is to determine the antioxidant activity of the bilberry extracts that are used in the medicine practice by a cathodic voltammetry method.
We consider the influence of water and alcohol bilberry extracts on the process of oxygen electroreduction. From these extracts the most activity relation to the process of cathodic oxygen reduction showed alcohol (40%) bilberry extract. It was also stated that the alcohol extract of bilberry has a greater antioxidant activity than other known antioxidants such as ascorbic acid, glucose, dihydroquercetin.
Thus, after consideration of a number of plant objects, we showed the possibility of applying the method of cathodic voltammetry for the determination of total antioxidant activity of plant material and identifying and highlighting the most perspective sources of biologically active substances (BAS), as well as the ability of identifying extractants that fully extract BAS from plant raw materials. The activity data of extracts of plant raw materials gives an opportunity of establishing an effective yield phytopreparation based on bilberry that has an antioxidant effect.

1. Introduction

Nowadays therapy that includes antioxidants (AO) is becoming more common in the treatment of diseases associated with the development of radical chain oxidation processes in the body cells [1-3]. The use of natural AO for the treatment and prevention of free radical pathology has several advantages, such as: the absence of side effects for most of them; the low toxicity even during the extended use; formulations based on them are available for patients, etc. Many individual substances that are contained in plant raw material are currently synthesized and produced by chemical-pharmaceutical industry. However, the plant raw material is still very relevant in the application because it contains a large set of biologically active substances (BAS), which stimulates the immune system of the human body, providing a stabilizing effect during the flow of many vital activity processes. In recent years the interest in the methods of biologically active substances isolation and their activity evaluation in natural objects is increasing. Based on them the variety of food additives and drugs that stimulate the metabolic processes in the body are created [4-6]. Thereby, there is a great interest in a perennial plant from the family of Vacciniaceae - Vaccinium Myrtillus.

In national medicine Vaccinium Myrtillus is widely used because of its astringent qualities due to the presence of tannins. Tinctures and extracts of the fruits are used in cases of acute and chronic

enterocolitis, putrid fermentation in the bowels, especially children, diarrhea, milder forms of diabetes. It externally applied as compresses for treating hemorrhoids. There are also indications of catarrhal and follicular tonsillitis, burns, canker sores, etc. treatment with the bilberry broth. Also the fruit of bilberries improves vision, especially night vision [7-9].

Thereby, the aim of the study is to investigate the influence of Vaccinium Myrtillus extracts on the electrochemical behavior of oxygen in water in the condition of the semi-infinite linear diffusion.

2. Methods and materials

We used a express and highly sensitive method of voltammetry (VA) having simple method implementation.

All experiments were carried out on a computerized voltammetric analyzer TA-2 produced by LLC Research and Production Enterprise RPE «Tomanalyt» (Tomsk) with three-electrode cell. Mercury-film electrode (MFE) was used as an indicator electrode, and argent chloride electrode served as an auxiliary reference electrode (ACE).

Direct cathode-current mode VA was used; speed of potential sweep (W) was 40 mV/s, working potential range from 0 to -1 V. The substances were mixed by vibration of the electrodes.

As a model reaction the oxygen reduction reaction was used. In the beginning we recorded the first wave of cathodic reduction of oxygen dissolved in a supporting electrolyte in the specified potential range. Then we successively added the solution of investigated substance and recorded the voltammograms of the first wave of oxygen cathodic reduction.

Oxygen reduction reaction was used as a model reaction. Records were in following order: record of first wave cathodic reduction of oxygen dissolved in a supporting electrolyte in the specified potential range. After substance addition, the solution was stirred about 20s. After the stirring is stopped, the potential was scanned negatively, causing oxygen reduction, giving a current first wave electroreduction of oxygen (ER O_2). Its value was proportional to the amount of oxygen in the bulk of the solution. Oxygen concentration was monitored by oxygen analyzer. Based on the ammetric measurements the concentration of oxygen in phosphate buffer at 25.0 ± 0.5°C was $2.56 \pm 0.05 \cdot 10^{-4}$ $mol \cdot l^{-1}$.

Oxygen concentration in the electrolyte solutions was monitored by potentiometric oxygen sensor № 5972 produced by the center of computing automation and measuring «MERA-ELVRO», with automatic temperature compensation in the range from 0 to 40 C. All reagents were of analytical grade. Samples material were weighted on laboratory analytical balance VL 210 firm «Gosmetr» with a weighing error of ± 0.0002 g. Nanopure water was used for making solutions.

3. Experimental

Recent studies have shown that herbal medicines contained a complex of biologically active substances have influenced on the various body systems, including antioxidant defense system [10-12].

The list of antioxidant activity (AOA) determination methods is quite wide: chemiluminescent method, gas phase chromatography, spectrophotometric and fluorometric methods, etc [13-15]. For this study we used cathodic voltammetry method to determine the antioxidant activity of vegetable raw materials [16]. The method is based on a process of oxygen electroreduction (ER O_2) that has a mechanism similar to the restoration of oxygen in the cells and body tissues.

In this study we used vaccinum myrtillis' shoots. Hydroalcoholic extracts of vegetable raw materials were prepared by mixing water and ethanol in various proportions. Different ratios of water and alcohol (40, 70 and 95 percent) in order to obtain extracts of vaccinum myrtillis' shoots were examined.

The influence of the test substances on the electrochemical behavior of oxygen is considered in a supporting electrolyte – phosphate buffer with pH 6.86 (0.025M KH_2PO_4 and 0.025M Na_2HPO_4).

The total antioxidant activity of vaccinum myrtillis' extracts was determined by the relative decrease in the current ER O_2 (figure 1) in the presence of the test components in solutions.

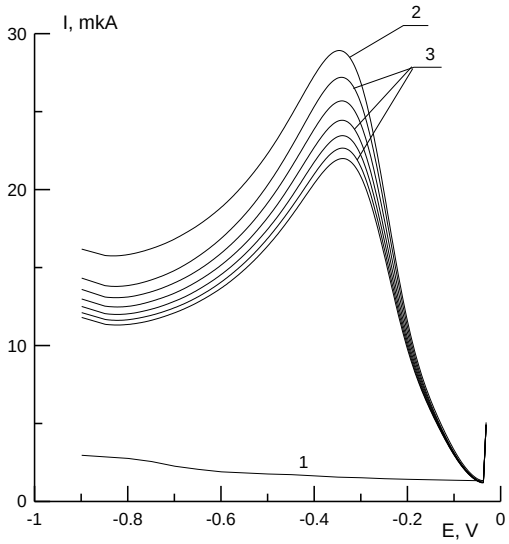

Figure 1. Voltammograms of the ER O_2 current in phosphate buffer (0.025M, pH 6.86) on the MFE without (2) and with 10^{-4} g/ml of vaccinum myrtillis aqueous extract in the solution (3), the residual current of the supporting electrolyte in the absence of O_2 and the substance in the solution (1). Potential scan rate is – 40 mv / s.

Lines (3) in figure 1 characterize the reduction of oxygen current with time of the reaction between the active oxygen forms and BAS vaccinum myrtillis extract in solution.

The dependence of relative changes of the limiting current ER O_2 on the time of the process in the presence of the sample was build according to obtained data. The kinetic criterion for characterizing the total AOA - K (μmol $l^{-1}min^{-1}$) was calculated with the help of the slope of linear part of the curve (Figure 2) [17].

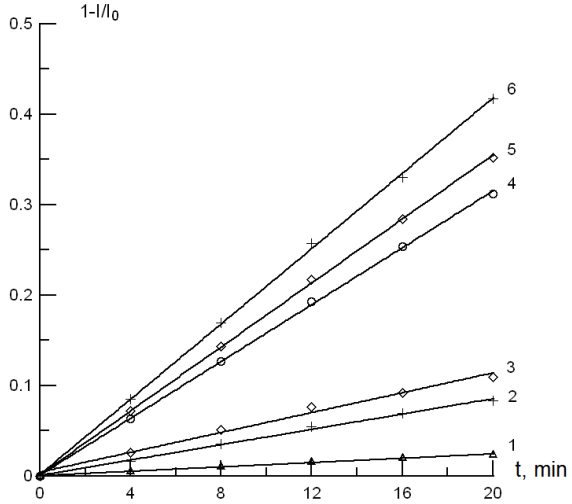

Figure 2. The linear part of the relative change of the limiting current of ER O_2 on the time of the process in the presence of 10^{-4} g/ml of vaccinum myrtillis extracts: water (1), 40% (6) 70% (4) and 95% (5) ethanol; dihydroquercetin (2), ascorbic acid (3).

4. Discussion of results

Experiments showed activity of all samples against of ER of O_2 process, but the activity of the water based samples are considerably smaller in comparing with alcoholic based samples which sufficiently close to each other in activity (table 1).

It is noteworthy that all samples moved the first wave potential of O_2 ER to a negative region, which means a loss of the thermodynamic process. The exception was the aqueous extract in the presence of its wave potential of ER O_2 shifted to a positive region, which gives the right make a conclusion about facilitating the process of ER O_2 by adding the sample solution in the background electrolyte solution. Such behavior of the cathode waves let us to suggest that in this case the probable EC mechanism of inhibition of the electrode process ER O_2 in the presence of AO includes subsequent chemical reaction of AO with active oxygen radicals.

Table 1. The total antioxidant activity of the vaccinum myrtillis extracts in relation to the process of ER O_2 (p=0.95, n=5).

Substance name	K (μmol $l^{-1}min^{-1}$)
The vaccinum myrtillis aqueous extract	0.153 ± 0.012
The vaccinum myrtillis extract with 40 % of ethanol	5.815 ± 0.026
The vaccinum myrtillis extract with 70 % of ethanol	4.351 ± 0.018
The vaccinum myrtillis extract with 95 % of ethanol	4.907 ± 0.037
Ascorbic acid	1.165 ± 0.053
Glucose	0.169 ± 0.037
Dihydroquercetin	0.650 ± 0.040

Thus the most active alcoholic extracts of vaccinum myrtillis is the extract with 40% of ethanol. It should be noted that the activity of considered ethanol extracts surpass the activity of such well known antioxidants like ascorbic acid and dihydroquercetin.

Study of the extracts effects on animals showed the highest activity of the extract with 70% of ethanol. In order to establish extractant that fully take out BAS there was a study of vaccinum myrtillis extract, obtained by treatment of 70% of ethanol. Fractionation was carried out by the extract with a number of solvents with increasing polarity. Due to this plant extract was dissolved in water (1:40) and extracted in a separating funnel sequentially with chloroform, ethyl acetate and butanol (table 2).

The first wave of ER O_2 was recorded in the presence of various fractions of the extract vaccinum myrtillis shoots with 70% of ethanol.

Table 2. AOA of vaccinum myrtillis extract shoots with 70% of ethanol and its fractions in the relation to the process of ER O_2 (p=0.95, n=5).

The sample	K, μmol $l^{-1}min^{-1}$
The chloroform fraction	3.432 ± 0.035
The ethyl acetate fraction	0.751 ± 0.014
The butanol fraction	5.604 ± 0.005
The aqueous residue	2.047 ± 0.005

The presented data show that the butanol fraction extract is the most active and the ethyl acetate is the most inactive. It can be said that butanol is best in removing of the bioactive substances from the extract of vaccinum myrtillis with 70% of ethanol.

5. Conclusion

Thus we showed the possibility of applying the method of cathodic voltammetry for the total antioxidant activity determination of vegetable raw materials, identification and selection of the most perspective sources of biologically active substances. We also demonstrated the possibility to identify extractants that fully extracting BAS from vegetable raw materials with a purpose of their concentration and the creation of pharmaceutical products based on them.

The obtained data on the activity of extracts of plant raw materials suggested in the paper give a possibility of creation of effective phytopreparation based on vaccinum myrtillis that has an antioxidant effect.

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