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STUDY OF MINT EXTRACTS ANTIOXIDANT ACTIVITY BY ELECTROCHEMICAL METHODS

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Abstract. The total amount of antioxidants and their activity with respect to oxygen and its radicals are measured in water extract of mint peppery (*Mentha piperita L.*) by two electrochemical methods: ammetric and voltammetric. Mechanisms of interaction of antioxidants with oxygen and its radicals have been investigated.

Keywords: antioxidant, total antioxidant activity, ammetric and voltammetric methods; oxygen radicals.

1. Introduction

Nowadays great attention is given to research of the quantity and activity of antioxidants (AO) in herbs, foodstuff, drinks, and other objects. It is known that increase in activity of oxidation processes in an organism leads to damage of structure and properties of lipid membranes. There is a direct dependence between the superfluous quantity of free radicals in organism and occurrence of dangerous diseases [1].

Antioxidants are biologically active substances, which remove excessive free radicals, decreasing the lipid oxidation. Therefore a detailed research of total antioxidant activity (TAA) and mechanisms of interaction with oxygen and its radicals are highly prospective. [2].

Mint peppery (*Mentha piperita L.*) is one of the most widespread types of herbs. It is widely used in medicine, cookery, and household, as well as pharmaceutical and cosmetic industry. Mint is applied as a medication in the form of infusions and teas for treatment of gastroenteric diseases, as demulcent at palpitation, depression and insomnia, in sedatives, as anesthesia at burns and insect stings, as well as analgesic and antistress medicine. Antioxidant properties of mint allow to prevent cataract and other illnesses connected with ageing of an organism. Mint peppery basic component (2–3 %) is menthol defining its taste and anesthetizing properties. Other substances contained are ethers, phellandrene, pinene, jasmole, piperitone, menthofuran, *etc*. There are also tannic and resinous substances, carotin (0.01 %), ascorbic acid (0.01 %), routines (0.015 %), and other polyphenolic compounds [3].

In the literature there are publications devoted to measurement of the AO quantity and activity in extracts of mint [4-7]. However, these are basically complex chemical methods, and the results received are poorly comparable among themselves, including the absence of uniform measuring units while the use of two operative electrochemical methods allows studying the antioxidant activity of mint after extraction.

2. Experimental

The purpose of the present work is to measure the antioxidant activity (AOA) of a water mint extract by a voltammetric method and to study the mechanisms of influence of mint components on the process of electrochemical oxygen reduction (ER O_2). Concurrently measurements of the total phenol antioxidant amount have been conducted by an ammetric method.

The object of the research was water extract of the mint peppery (*Mentha piperita L.*), collected and dried in 2008 year in Tver region (Russia). Dry herb was grinded in a mortar till particles of the size 1–2 mm were obtained. Further this herb (0.5 g) was immersed into 50 ml of distilled water with T = 318 K and held for 10 min without thermostating. Then the extract was carefully filtered through a paper filter “a dark blue tape” and if necessary diluted before measurements [7]. Acidity of the extract (pH) has been defined as 6.6.

In the present work two electrochemical methods were used. The ammetric method [8] allows defining the total phenol type antioxidant amount in investigated samples. Measurements were conducted on the device “Zwet-Jauza-01-AA” [9]. Electric current of oxidation of

the investigated substance (or mixes of substances) on a surface of a working electrode at constant potential +1.3 V was measured. Only OH-groups of natural phenolic type antioxidants are oxidized under this potential (+1.3 V) [7–9]. The received signal from the tested sample is compared with a signal of an individual antioxidant – gallic acid, measured under the same conditions. The measurement error is not more than 10 %, measurement time is 10–15 min [9].

The voltammetric method uses the process of oxygen electroreduction (ER O_2) as modeling reaction. This process is similar to oxygen reduction in tissues and plant extracts. It proceeds at the working mercury film electrode (MFE) in several stages with formation of the reactive oxygen species (ROS), such as $O_2^{\cdot -}$ and $HO_2^{\cdot}$ [10]:

$$O_2 + e^- \rightleftharpoons O_2^{\cdot -} \qquad E^o = +0.012 \text{ V} \quad (1)$$

$$O_2^{\cdot -} + H^+ \rightleftharpoons HO_2^{\cdot} \quad (2)$$

$$HO_2^{\cdot} + H^+ + e^- \rightleftharpoons H_2O_2 \qquad E^o = +0.682 \text{ V} \quad (3)$$

$$H_2O_2 + 2H^+ + 2e^- \rightleftharpoons 2H_2O \quad E^o = +1.228 \text{ V} \quad (4)$$

For determination of total antioxidant activity in work [10] it is offered to use the first wave of ER O_2 corresponding to stages (1)-(3) when on a surface of a mercury film electrode active oxygen radicals and hydrogen peroxide are formed. It should be noted that antioxidants of various natures were divided into 4 groups according to their mechanisms of interaction with oxygen and its radicals (Table 1) [11].

The first group of biologically active substances increased oxygen current resulting in catalytic effect on the kinetics of the ER O_2 *via* reaction of hydrogen peroxide disproportion and partial regeneration of molecular oxygen according to the mechanism (5)-(7).

$$O_2 + e^- + H^+ \rightleftharpoons HO_2^{\cdot} \quad (5)$$

$$HO_2^{\cdot} + e^- + H^+ \rightleftharpoons H_2O_2 \quad (6)$$

$$2H_2O_2 \xrightarrow{\text{catalyst}} 2H_2O + O_2 \quad (7)$$

For the second group of the antioxidants we suggest the following mechanism of interaction of antioxidants with the reactive oxygen species (ROS) (8):

$$O_2 + e^- \underset{}{\overset{k_0}{\rightleftharpoons}} O_2^{\cdot -} + \text{R-OH} + H^+ \overset{k_1^*}{\rightleftharpoons} H_2O_2 + \text{R=O} \quad (8)$$

The third group of the BAS decreased oxygen current with shift of the ER O_2 potential to the negative range. Reaction between the BAS and oxygen and its species (ROS) can be represented *via* the following mechanism (9)-(11).

$$O_2 \longrightarrow O_2^S \underset{k_1}{\overset{+RSH}{\longrightarrow}} HO_2 + \bar{e} \overset{k_{R1}}{\rightleftharpoons} HO_2^{\bullet -} + RS^{\bullet} \quad (9)$$

$$HO_2^{\bullet -} + RSH \rightleftharpoons H_2O_2 + RS^{\bullet} \quad (10)$$

$$RS^{\bullet} + RS^{\bullet} \rightleftharpoons RS - SR \quad (11)$$

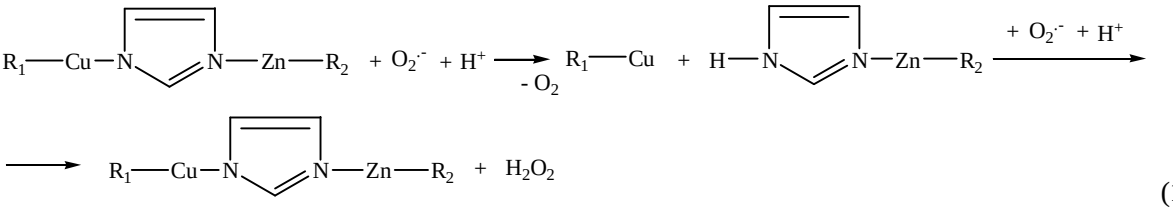
Table 1

Groups of biologically active substances (BAS) divided according to mechanisms of interaction with oxygen and its radicals

Number of groups	1 group	2 group	3 group	4 group
Substance names	Catalysts, phtalocyanines of metals, humic acids	Phenol nature substances, vitamins A, E, C, B, flavonoids	N, S, Se-containing substances, amines, amino acids	Superoxide dismutase (SOD), porphyry metals, cytochrome C
Influence on ER O_2 process	Increase in current of ER O_2, potential shift in negative area	Decrease of current of ER O_2, potential shift in positive area	Decrease of current of ER O_2, potential shift in negative area	Increase in current of ER O_2, potential shift in positive area
The prospective electrode mechanism	EC* mechanism with the following reaction of hydrogen peroxide disproportion and partial regeneration of molecular oxygen	EC mechanism with the following chemical reaction of interaction of AO with active oxygen radicals	CEC mechanism with chemical reactions of interaction of AO with oxygen and its active radicals	EC** mechanism with catalytic oxygen reduction via formation of intermediate complex

Note: * E – electrode stage of process, C – chemical reaction.

For the fourth group of the BAS we suggest the mechanism with catalytic oxygen reduction *via* formation of intermediate complex similar by SOD (12).



(12)

Kinetics criterion K is used as an antioxidant activity criterion of the investigated substances. It reflects quantity of oxygen and their radicals which have reacted with AO (or their mixes) in a minute (13, 14).

For the second and the third groups of antioxidants:

$$K = \frac{C_0}{t}(1 - \frac{I}{I_0}), \text{ µmol/l·min}, \quad (13)$$

For the firth and the fourth groups of antioxidants:

$$K = \frac{C_0}{t}(1 - \frac{I_o}{I}), \text{ µmol/l·min}, \quad (14)$$

where I, I_0 are limited values of the current of oxygen electroreduction in the presence and the absence of AO in the supporting electrolyte, correspondingly; C_0 is initial concentration of oxygen in the supporting electrolyte (µmol/l), *i.e.* solubility of oxygen in supporting electrolyte under normal conditions; t is the time of interaction of antioxidants with oxygen and its radicals in min.

For voltammetric study of the total antioxidant activity of the samples automated voltammetric analyzer "Analyzer of TAA" (Ltd. "Polyant" Tomsk, Russia) was used. As supporting electrolyte 10 ml of phosphate buffer (pH = 6.76) with known initial concentration of molecular oxygen was used [10]. An electrochemical cell (V = 20 ml) was connected to the analyzer and consisted of a working mercury film microelectrode (MFE), a silver-silver chloride reference electrode with KCl saturated ($Ag|AgCl|KCl_{sat}$) and a silver-silver chloride auxiliary electrode. The area of surface of MFE is very small (S = 0.40 mm^2) compared to the area of reference electrode (S = 5.28 mm^2) causing minimum effect of polarization of the working electrode. An open type cell should be used in this investigation. The reference electrode and the working electrode were held in the electrochemical cell. The working electrode potential was initially set at 0 V for about 120 s. During this step the solution was stirred by a magnetic stirrer. After stirring the potential was scanned negatively, causing oxygen reduction, which gives the first current wave at E = (-0.1)–(-0.3) V. Its value is proportional to the amount of oxygen in the solution bulk. Potential rate scan was 0.1 V/s, potential range was E = 0 –(-0.9) V. The voltammetric method of research has good sensitivity. It is simple and cheap.

3. Results and Discussion

In Figs. 1-3 the voltammograms of the oxygen electroreduction without and with the investigated samples, received at various times after mint extraction are resulted: after extraction t = 5 min (Fig. 1), t = 30 min (Fig. 2) and t = = 60 min (Fig. 3). One can see that the fresh mint extract (Fig. 1) shows the mechanism of phenol nature AO, reducing the oxygen current and shifting potential in positive area (2nd group in the Table). The kinetics criterion K according to (13) grows to values 2.0±0.5 µmol/l·min in 30 min after extraction.

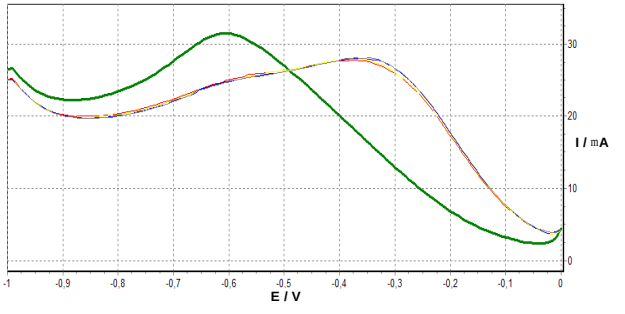


Fig. 1. Voltammogram of the ER O_2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (left curve) and in the presence (right curve) of mint extract at t = 5 min after mint extraction.
Extract dose d = 100 µl

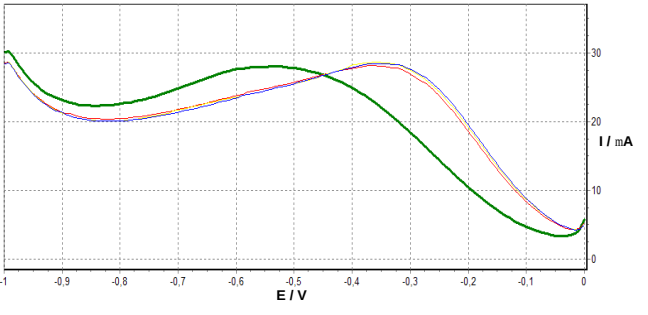


Fig. 2. Voltammogram of the ER O_2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (left curve) and in the presence (right curve) of mint extract at t = 30 min after mint extraction.
Extract dose d = 100 µl

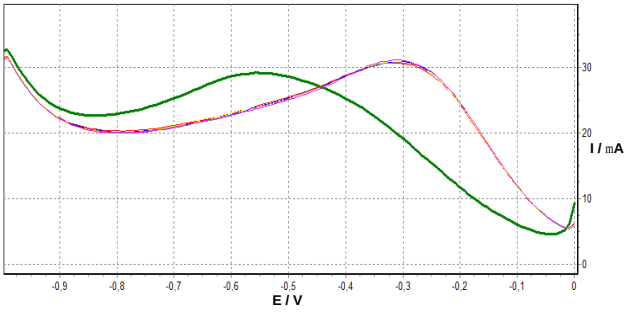


Fig. 3. Voltammogram of the ER O2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (left curve) and in the presence (right curve) of mint extract at t = 60 min after mint extraction. Extract dose *d* = 100 µl

However, approximately in an hour after extraction (Fig. 3), character of interaction of components of mint with oxygen and its radicals varies, following the mechanism characteristic of the substances of the 4th group (see Table 1). Transition from one mechanism to another occurs approximately during 30 min after extraction (Fig. 2) and the kinetics criterion *K* becomes thus close to zero. Shift of potential of the current half wave of oxygen redu-ction (Δ) remains approximately identical (0.20–0.25 V).

During the experiment the influence of extract dose *d* in supporting electrolyte on the kinetics criterion *K* and on the shift of potential of the current half wave of oxygen reduction (Δ) was investigated. The results show that *K* and Δ decrease from *K* = 1.5±0.5 µmol/l·min, Δ = 0.25 V for *d* = 100 µl (Fig. 3) to *K* = 1.3±0.3 µmol/l·min and Δ = 0.10 V for *d* = 25 µl (Fig. 4) and *K* = 0.8± ±0.2 µmol/l·min, Δ = 0.05 V for *d*=10 µl (Fig. 5).

In parallel for the same mint extract the registration of the total phenolic antioxidant amount *C* (in units of gallic acid) has been measured from an extract storage time *t* by ammetric method (Fig. 6). Dependence *C* on *t* testifies to notable falling of *C* after extraction and filtering of a mint extract (approximately 20 % for 2 h of storage). It is possibly connected with destruction of the unstable phenol substances contained in an extract. Therefore, apparently, voltammogram character and values of kinetics criterion (Figs. 1-3) during the first moment after extraction could be established as influence of classical phenolic antioxidant on the process of electroreduction of oxygen and its radicals.

As it has been mentioned in the introduction, one of the basic components of mint extract is menthol $C_{10}H_{20}O$ [12], therefore voltammogram of ER O_2 with menthol has been studied at supporting buffer. Menthol (60 mg) has been dissolved in 100 ml of hot water and 200–500 µl were added in 10 ml of the buffer. Voltammograms have been obtained under the same conditions as for the mint extract.

In Fig. 7 a typical example of such voltammogram is presented. One can see that menthol "works" as classical AO, decreasing the current of oxygen electroreduction. For the dose of the menthol solution *d* = 500 µl the value of kinetics criterion *K* was 1.5±0.4 mcmol/l·min, slowly decreasing at reduction of menthol concentration.

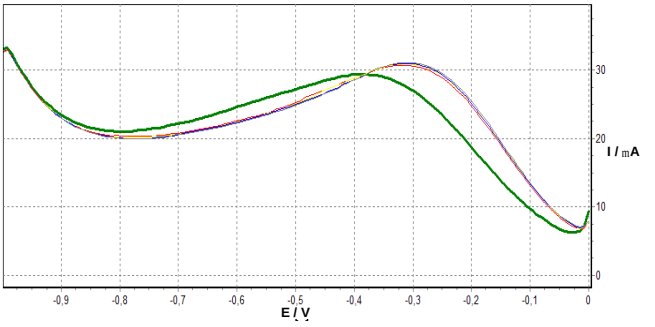


Fig. 4. Voltammogram of the ER O2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (left curve) and in the presence (right curve) of mint extract at *t* > 60 after mint extraction. Extract dose *d* = 25 µl

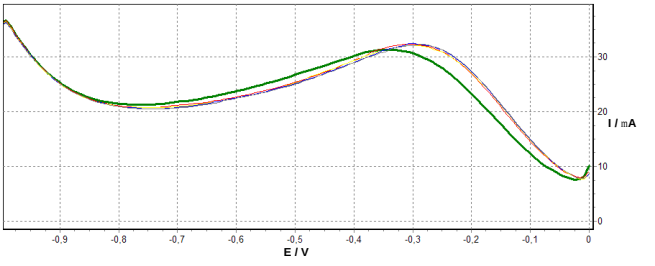


Fig. 5. Voltammogram of the ER O_2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (left curve) and in the presence (right curve) of mint extract at *t* > 60 after mint extraction. Extract dose *d* = 10 µl

Such behavior was also observed for cyclohexanol (similar menthol substance) whose activity at the same concentration was included into the same interval of values *K*, as for menthol under the same conditions. Voltammograms for such components of mint as routines and ascorbic acid are a classical example of the 2nd group of antioxidants from Table 1 [13].

4. Conclusions

It can be concluded that the substances of phenolic type (2nd group of BAS in Table 1) define the mechanism of interaction of mint components with oxygen and its radicals during 30 min after extraction. At further storage of an extract these unstable substances are destroyed and the mechanism of interaction of mint components with oxygen and its radicals is changed to the one typical of the 4th group of the BAS in Table 1. Probably, metal complexes as mint components are dominated causing increase of ER O_2 current and catalytic mechanism of oxygen electro-reduction.

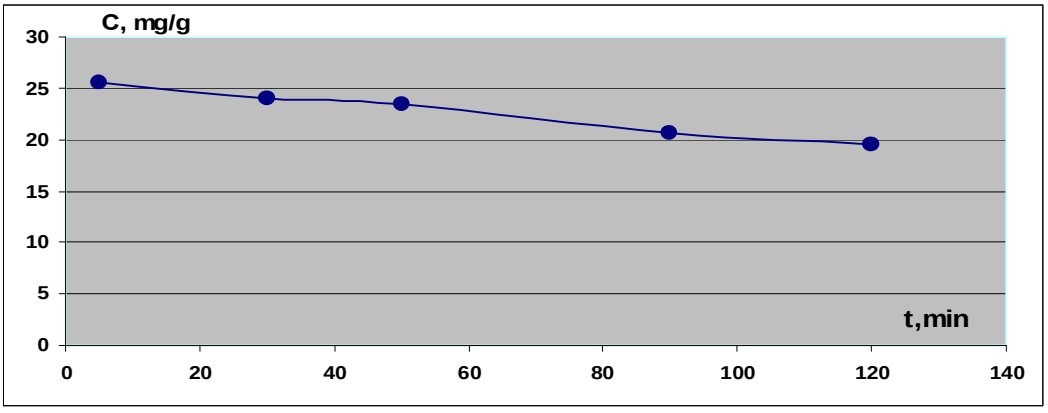


Fig. 6. The total phenol antioxidant amount *C* in a mint extract depending on extract storage time *t*

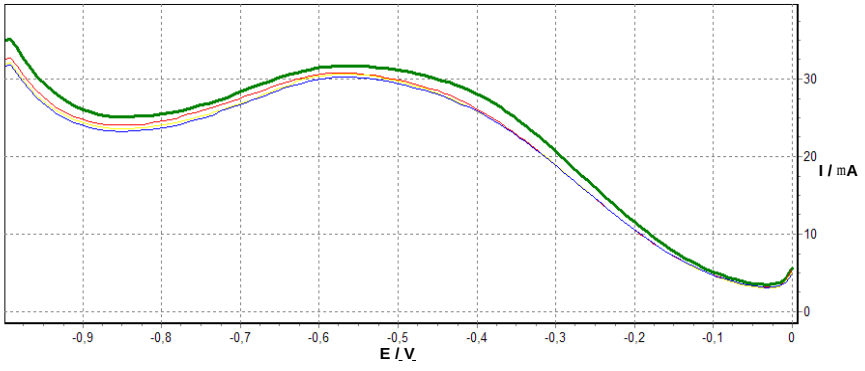


Fig. 7. Voltammogram of the ER O_2 current in the supporting electrolyte 0.025 M phosphate buffer (pH = 6.86) in the absence (upper curve) and in the presence (bottom curve) of water menthol solution. Menthol solution dose *d* = 500 µl

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ВИВЧЕННЯ ЕЛЕКТРОХІМІЧНИМИ МЕТОДАМИ АНТИОКСИДАЦІЙНОЇ АКТИВНОСТІ ЕКСТРАКТІВ М'ЯТИ

Анотація. *За допомогою двох електрохімічних методів (амперометричних і вольтамперометричних) визначено у водному екстракті м'яти перцевої (Mentha piperita L.) загальну кількість антиоксидантів та їх активність по відношенню до кисню і його радикалів. Досліджені механізми взаємодії антиоксидантів з киснем та його радикалами.*

Ключові слова: *антиоксиданти, загальна антиоксидаційна активність, амперометрія, вольтамперометрія, кисневі радикали.*