MEDICAL SCIENCES

POTENTIOMETRIC DETERMINATION OF THE ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS

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Abstract. The article deals with the analysis of the existing methods of investigating the antioxidant activity of plants' origin and the expediency of measuring oxidation-reduction potential for this purpose.The integral AOA of alcohol extracts *Urtica dioica, Trifolium pretense, Chelidonium majus, Hippophae rhamnoides* has been determined by potentiometric method using the mediator system. It was established that the leaves of sea – buckthorn are characterised by the highest antioxidant activity (2.05 ± 0.1 mg AA / ml). The AOA of plants *Urtica dioica, Chelidonium majus,* that were growing along the transport zone is higher by 23,1-27,9% comparing with the plants collected from environmentally friendly area. It was found out that the plants of *Hippophae rhamnoides* L. coriander were adapted to oil pollution, as evidenced by the absence of a significant difference between AOA of plants collected from environmentally friendly area comparing with the plants that were growing on the soil polluted with petroleum products.

Keywords: antioxidants, medicinal plants, potentiometric method, integral antioxidant activity.

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Introduction

It is believed that the accumulation of free radicals in the human body is one of the main reasons for the develop of such serious diseases as malignant neoplasms, chronic inflammatory processes, diabetes mellitus, neurological pathologies, atherosclerosis, ischemic heart disease, heart attack, stroke *(Khan, 2012).* The concentration growth of free radicals

increases as a result of radiation, UV radiation, smoking, alcoholism, constant stress, infectious diseases, inproper nutrition, as well as due to the decrease in the activity of the natural antioxidant system of humans.

Problems of chemical regulation of oxidative stress and the search for biologically active substances that possess an antioxidant activity (AOA) have become the subject of many investigations *(Zenkov et al, 2001).* The best sources of antioxidants are plants, in particular the medicinal ones, due to their content of biologically active substances – phenolic compounds (flavonoids, flavonols, catechins, etc.), vitamins (C, E), carotenes, minerals *(Papastergiadis et.al., 2012).* Natural antioxidants, as a rule, suppress the reaction of free radical oxidation by binding free radicals and the formation of stable chemical compounds, thereby creating optimal conditions for metabolism and ensuring the normal growth of cells and tissues.

Methods of the research of total antioxidant capacity (TAC) differ in type of source of oxidation, oxidizing compound and method of measuring its concentration *(Polumbryk et al., 2016).* Methods of measuring antioxidant activity are divided, depending on the form of registration, on the methods of optical spectrometry (fluorimetric, chemiluminescent, photocolormetric, spectral), electrochemical (amperometric, voltamperometric, potentiometric), specific – chromatographic, titrimetric, biochemical analysis, electronic paramagnetic resonance (EPR), EPR with spin traps, mass spectrometry, etc. All methods have advantages and disadvantages *(Amorati et.al., 2015).*

The most numerous methods and modifications of the methods mentioned in the literature, use photometric registration, perhaps, as the most convenient and accessible. In particular, there are common methods which are based on the overall reduction effect of individual low molecular weight antioxidants by color change relative to the standard compound, which is a water soluble analogue of vitamin E – Trolox *(Harasym et al., 2014; Lucio et al., 2009).* Antioxidant activity in brain tissues was investigated with Trolox as an equivalent (and named TEAC - trolox equivalent antioxidant capacity) *(Chatterjee et al., 2005).*

Also, a method for oxidizing deoxyribose in a radical system is described in the literature sources *(Choi et.al, 2014)*. Another photometric method is based on photocolorimetry of iron-thiocyanate complexes (Nilsson J. et al., 2006). The classical methods for evaluating the antioxidant activity of natural compounds are the study of the kinetics and their interaction with stable radicals, in particular, 2,2-diphenyl-1-pyrilhydrazil DPPH) and 2,4,6-triphenyl-verdazyl (TPV) *(Choi et.al, 2014).*

AOA can be estimated by inhibition of the accumulation of finite molecular products of free radical oxidation, in particular malonic dialdehyde. The interaction with 2-thiobarbituric acid with the formation of a colored complex is used for its quantitative determination *(Papastergiadis et.al., 2012).*

Recently, a significant role of active forms of nitrogen oxide NO has been noted in the pathology of various diseases, for example, ischemia *(Zhang et al., 2015).* The reaction of inhibition of the forming active forms of nitrogen oxide is used to evaluate the antioxidant properties of the compounds with the help of ascorbic acid by measuring the optic density *(Toth et al., 2002).*

Nowadays, one of the most widely used methods is the method of determining adsorption capacity in relation to oxygen-containing radicals ORAC (oxygen radical absorption capacity) *(Martin I. et al., 2009).* This method is based on measuring the fluorescence intensity of a certain compound (more often fluorescein) and its change from the

duration of the reaction. In the presence of compounds that bind oxygen-containing radicals, the time of fluorescence increases as a result of the protective effect of antioxidants *(Amorati et al., 2015).*

In the research work *(Trindade et.al., 2016)* has been proposed a quick method of evaluating the antioxidant activity of fruit and vegetable extracts directly in living mammalian cells. It consists in the use of oxidation of 2 ', 7'-dichlorofluorescein acetate (DCFH-DA) (indicator of reactive compounds of oxygen) for determination of pro- and antioxidant capacity of the individual compounds as well as fruits and vegetables *(Trindade et al., 2016).* Several methods have been used to determine the content of phenolic compounds and (+) - catechins in various types of chocolate (18 samples) *(Hu et al., 2016).* Most of the chocolate polyphenols belong to the class of flavonoids, which determine the high antioxidant activity of chocolate as compared with black and green tea, as well as red wine.

Accordingly, the most common methods are such as TEAC (trolox equivalent antioxidant capacity), total radical trapping antioxidant (TRTAP), FRAP (ferric reducing antioxidant power), etc., based on the reaction of the reduction of long-lived free radicals or the Fe(III) complex.

The main defect of these methods is the fact that antioxidant activity is considered in them as a function of many parameters (in particular, time, temperature, nature of substance, concentration of antioxidant and other compounds); during the implementation of these methods, synthetic free radicals are used, which have nothing in common with free radicals in the human body *(Polumbryk et al., 2016).*

The interaction of antioxidants with free radicals and active forms of oxygen in the aqueous medium is accompanied by the passing of an electron, and, consequently, has an electrochemical nature. Therefore, for the purpose of determining the integral antioxidant activity of plant material, it is advisable to use potentiometric methods that are characterized by high sensitivity, simplicity of execution, informative and affordable value *(Sharafutdynova et al., 2004).*

The purpose of the study was to explore the antioxidant peculiarities of the medicinal plants of common *Urtica dioica, Trifolium pretense, Chelidonium majus, Hippophae rhamnoides* by potentiometric method.

Materials and methods of the research

The testing materials was the following: grass of the common *C. majus*, the leaves of *H. rhamnoides*, *U. dioica*, the grass of the meadow clover *T. pratense*, which grew on provisionally clean area along the transport zone and ozokerite dumps. Leaves and herbs were harvested during mass flowering. *U. dioica* and *T. pratense* were harvested in June – July, *C. majus* – in May, *H. rhamnoides* – in early August. Flowering tops of 10-15 cm in lenght were cut in dry *T. pratense* weather in the morning after dew droping. Desiccation was carried out in shaded areas with good ventilation at a temperature of 40 – 60 °C. The *C. majus* grass was dried quickly to prevent alkaloids and other physiologically active substances from disclosing. The first day's desiccation, were carried out in the sun and then continued in the dryer at a temperature of 55 – 60 ºC.

For further research, vegetative material was ground on a laboratory mill at 1000 rpm. to linear sizes of 5 mm in accordance with the requirements of the State Pharmacopoeia of Ukraine (SFU) *(Pharmacopoeia, 2008).*

Alcohol extractors were prepared using 70% alcohol in the ratio of 1:10 and further infusion for 14 days. After this, the extract was drained through a five-layer sterile gauze, the remaining raw material was pressed, washed with a small amount of extractant, again pressed and extracted with a filtered extract to the required volume.

The potentiometric method using the mediator system *(Lupak et al., 2017)* was carried out by the determination of AOA *(Lupak et al., 2017)*, modifying the methods of Braine and co-workers *(Brainina et al., 2004)* and Aronbayev and co-work *(Aronbaev et al., 2015)*. To measure the oxidation-reduction potential (ORP), the pH-150MI brand was used. A platinum electrode of the brand EPL-02 was used as an indicator, and the chlorine silver electrode of the mark EVL-1M3.1, filled with potassium chloride solvent with a concentration of 3 mol / dm^3 as a comparison electrode.

The mediator system consisted of solvents of K3 [Fe (CN) 6] and K4 [Fe (CN) 6] in phosphate buffer at pH 7,2 *(Brainina et al., 2004).* Initially, the potential of the platinum indicator electrode in the mediator system was measured. The extract of 0.2 ml of the test solution was mixed with 10 ml of the mediator to determine the AOA. After determining the equilibrium, the potential of the platinum indicator electrode was determined in the resulting mixture.

As a standard, AOA used freshly prepared alcoholic solutions of ascorbic acid (AA) at a concentration of 1, 2, 4 mg / ml in order to calibrate them for further investigation of the alcoholic extracts of the examined medicinal plant material *(Lupak et al., 2017).* Recalculation of AOA was done relative to the concentration of AA, because it is a known antioxidant.

In order to calculate the AOA, a calibration graph of the dependence of the difference in the potential of the mediator system before and after adding of a solvent of AA from the concentration logarithm AA in the initial standard solution for the alcoholic solvent AA was constructed (Fig. 1). The calibration was carried out for each subsequent series of extracts.

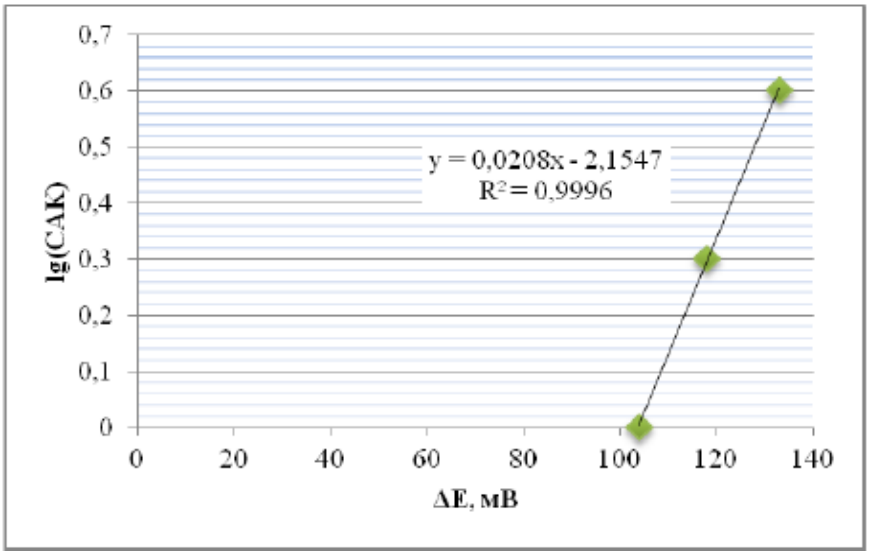

Fig.1 Calibration graph of the mediator system dependence for determination of AOA alcoholic solution of AA

The quantity of the antioxidant activity of AOAx were calculated for the investigated extracts, according to the general formula for the obtained gauge dependencies (2):

$$\lg[\ C(AA)\ ] = A \cdot \Delta E + B, \quad (1)$$

where A, B – coefficients of gauge dependencies, according to the least squares method, which is implemented in Microsoft Office Excel table processor; ΔE is the difference between the ORP of the mediator system before and after adding a solvent in which the AOA was measured.

Thus, AOAx in units of concentration of AA (mg / ml) in the extract was determined by the formula (2):

$$AOA_x = 10^{\lg[C(AA)]}, \text{ [mg AA/ ml]} \quad (2).$$

Statistical data was processed using Microsoft Office Excel, the dissimilarity between the samples were considered reliable at $p \leq 0.05$.

Results of the research and their discussion

In the result of extracts investigation of the grass of *C. majus*, leaves of *U. dioica*, *H. rhamnoides*, grass of *T. pratense*, it was found that the largest AOA is characterized by extracts of *H. rhamnoides* leaves (Table 1).

Table 1

AOA characteristics of alcoholic extract of plant material

(M±m, n=5)

Title of raw materials	Potential difference, (ΔE), мВ	lgC(AA)	AOA, mg AA / ml
Leaves *Urtica dioica*	110,7	0,15	1,41±0,06
Grass *Trifolium pratense*	109,2	0,12	1,31±0,07
Grass *Chelidonium majus*	109,7	0,13	1,34±0,06
Leaves *Hippophae rhamnoides*	118,6	0,31	2,05±0,1

The AOA quantity of H. rhamnoides leaf extract is 2.05 ± 0.1 mg of AA / ml, which is significantly higher ($p<0.01$) than AOA extracts of other investigated plants (by 45.4 – 56.5%).

The consequence of anthropogenic activity is environment pollution, including soils. It is known that technogenically polluted soils cause significant changes in the activity of the antioxidant plant system. In particular, along the roads where traffic is intense, with the exhaust gases of cars, a lot of the most widespread and most dangerous pollutants (environmental pollutants) such as – cadmium and plumbum gets to the soil surface. In this case, the mechanisms of adaptation to their toxic effects, which consist in the activity growth of some enzymes, in particular catalase, peroxidase, elevated polyphenol synthesis, ascorbic acid and enzymes of its metabolism are activated in plants.

In order to study the influence of soil pollution on the antioxidant state system of plants, AOA alcoholic extract of grass *C. majus* and *U. dioica* leaves, which grew on conditionally clean territory and along the transport zone were determined. It was found that

AOA was significantly higher (p <0,05, t = 3,10) above 23,1% in extracts of grass *C. majus*, collected from an technogenically polluted area for the extracts of *U. dioica* leaves, a similar pattern was observed: AOA was significantly lower (p <0,05, t = 2,72) above 21,9% in extracts of plants collected on a conditionally clean area (Table 2).

Table 2

AOA characteristics of herbs extractors *C. majus* and *U. dioica* depending on their growth conditions

(M ± m, n = 5)

Raw materials growth place	**AOA *C. majus*, mg AA / ml**	**AOA *U. dioica*, mg AA / ml**
Conditionally clean area	1,34±0,06	1,41±0,07
Along the transport lane	1,65±0,08	1,72±0,09

Consequently, AOA grasses *C. majus* and *U. dioica* leaves grow in conditions of anthropogenic loading on the soils where they grow on.

It is known from the literary sources that the sea-buckthorn improves the soil, due to its ability to form a powerful forest litter, enriched with nitrogen, phosphorus, potassium and organic matter. The root system of the plant is capable of symbiosis with microorganisms of the soil, which results in successful overgrown areas contaminated with oil in the process of phytoremediation. Excess heavy metals in oil-contaminated soils accumulate a little in plants of sea-buckthorn during long-term growth on these soils. Under such conditions, the protection mechanisms of sea buckthorn are launched, as a result of which the physiological adaptation of plants develops and they can withstand stressful influences *(Shevchyk et al., 2017)*.

Taking into account these data, AOA in the leaves extracts of sea-buckthorn, which contains a lot of antioxidants that react quickly to stress factors, was investigated.

The raw material of plants growing on conditionally clean territory and on ozokerite dumps (on soils contaminated with oil) was selected for the research. Analyses results showed that the difference between the AOA extracts of plants collected on a relatively clean and contaminated oil territory was not significant (Table 3).

Table 3

AOA characteristics leaves hood of *Hippophae rhamnoides* depending on conditions of their growth

(M ± m, n = 5)

Raw materials growth area	**Potential differences, (ΔE), мВ**	**lgC(AA)**	**AOA, mg AA / ml**
Conditionally clean area	118,6	0,31	2,05±0,1
Ozokerite dumps	116,9	0,26	1,89±0,09

The obtained results confirm the data of literary sources about the ability of *H. rhamnoides* adaptations to oil pollution in the conditions of long-term influence.

Conclusions

1. The integral antioxidant activity of alcoholic extracts of *U. dioica, T. pretense, C. majus, H. rhamnoides* was determined by potentiometric method and found to be within the range of 1.31 ± 0.07 – 2.05 ± 0.1 mg AA / ml.

2. It was established that the highest antioxidant activity (2.05 ± 0.1 mg AA / ml) are characterized by extracts of *H. rhamnoides* leaves.

3. It was shown that the antioxidant activity of alcohol extracts of *C. majus* herb and the leaves of *U. dioica*, which grew along the transport lane, was higher by 23.1 – 27.9% as compared with the plants collected from an environmentally friendly area. This indicates changes in the antioxidant system activity of plants as a result of the oxidative stress caused by heavy metals that accumulate from the exhaust gases of transport.

4. It was found that there is no reliable difference between AOA alcohol extracts of *H. rhamnoides* leaf, collected from environmentally friendly and areas polluted with oil, which is proof of adaptation of the plant to oil pollution in the conditions of long-term influence.

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