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ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS

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On the example of the model reaction of cumene initiation oxidation on kinetic method, the antioxidant properties of ethyl acetate extracts of leaves and fruits of twelve different plants are investigated. Both the content of the antioxidant substances in the studied extracts and their antioxidant activity are defined. It is established that most of the studied extracts of antioxidants contained extracts of Evening Primrose leaves ($2.93\cdot10^{-5}$ *mol*/*L* in 1 *mg*) and Scarlet amaranth ($1.15\cdot10^{-5}$ *mol*/*L*). The highest antioxidant activity of the extracts from the leaves showed Coneflower ($3.21\cdot10^{5}$ *L*/*mol*·*s*), Pumpkin ($2.93\cdot10^{5}$ *L*/*mol*·*s*), Goosefoot ($2.70\cdot10^{5}$ *L*/*mol*·*s*) and Grecian Silk Vine ($2.58\cdot10^{5}$ *L*/*mol*·*s*). It was shown that oxidation products (*Q*) of origin antioxidants in the extracts also possess antioxidant activities. Correlation was found between the pre-exponential factor (lg*A*) and energy activation (*E*) for the constant rate reaction $RO_2^{\bullet} + InH \xrightarrow{k_7} ROOH + In^{\bullet}$ and $RO_2^{\bullet} + Q \xrightarrow{k_{71}} Q^{\bullet} + ROOH$. For constant k_7 is established that $\lg A = 4.9 + 0.64\,E$ and for k_{71} $\lg A = 2.6 + 0.64\,E$.

***Keywords*:** plant extracts, antioxidants, antioxidant activity.

Introduction. In the last decade due to environmental ecological degradation more attention is paid to the prevention of many diseases, the development of which somehow is associated with damaging effect of free radicals. In this regard natural plant antioxidants (AO) occupy a special place, which are characterized by low toxicity, easily extracted and have multifunctional activity [1–3]. Consequently, the search and study of plant extracts that have the highest antiradical and antioxidant activity (AOA) is a very urgent task.

Numerous works devoted to antioxidant activity of plant extracts are published. In these works AOA are given in units % *mg*, *mg*/*mL* or *mg*/*g*, that characterizes AO content in studied extracts [4–9]. From classical literature [10] it is known that AOA is a reaction rate constant of linear chain termination reactions to radical inhibitors (InH), in the same process of oxidation to rate reaction constant (k_7):

$$\text{I.}\quad RO_2^{\bullet} + InH \xrightarrow{k_7} ROOH + In^{\bullet},$$

which depends on the chemical composition of InH and it does not depend on the number of its contents.

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Antioxidant action, as inhibitors, so the plant extracts, are characterized by three parameters:

1. Content: the more AO content in the studied extract the longer deceleration time of radical process;

2. Capacity: shows the number of radicals terminating in one AO molecule. This parameter of AO in the extracts are not determined, because, extracts contain a chemical composition of two or more AO substances, usually which can lead both to synergism and to antagonism;

3. Activity: as was mentioned above, the AOA is characterized by constant rate reaction (I). For extracts AOA is an effective value, because, in the reaction (I) two or more inhibitor compounds are involved.

This study presents the results of determining the total content of AO in extracts of twelve plants and their antioxidant activities.

Experimental Part. The inhibition of cumene oxidation as a model reaction was selected. A manometric device with automatic pressure control was used to carry out the oxidation experiments.

Cumene, chlorobenzene, ethyl acetate and azobisisobutyronitrile (AIBN) are used as reagents and were purified according to the procedure described in [11]. The studied plant extracts were prepared as follows: after collection of the stuff they were dried in an oven at 313.15 *K*, then the dried stuff was milled in a ceramic mortar to a powder-like state (<1 *mm*), distilled ethyl acetate was added at room temperature (20 *mL* for 1*g* of the powder), then it was settled during a day and was filtered with a paper filter. The filtrate has been evaporated to constant weight at room temperature. The source of free radicals is AIBN, chlorobenzene was used as solvent.

The method is based on the direct detection of absorbed oxygen as result of cumene oxidation and the total content of AO in the studied extracts is determined by the detection of induction periods (τ):

$$\tau = f \cdot [\mathrm{InH}] / W_i, \qquad (1)$$

where W_i is the initiation rate; f is the capacity; AO is the stoichiometric inhibition factor; [InH] is the total concentration of AO in the studied extracts. As we did not measure the parameter f, the product f·[InH] has been taken for total content of AO.

The studied extracts AOA were determined straightening the experimental data in the coordinates of the equation

$$[\mathrm{O_2}] = -(k_2/k_7)\,[\mathrm{RH}]\,\ln(1-t/\tau), \qquad (2)$$

where $[\mathrm{O_2}]$ is the amount of oxygen absorption during $t < \tau$, k_2 is the chain continuation reaction rate constant [12]:

$$\text{II.}\quad \mathrm{RO_2^{\bullet} + RH \xrightarrow{k_2} ROOH + R^{\bullet}},$$

where [RH] is the concentration of oxidized hydrocarbon, i.e. cumene. In all experiments [RH] = 2.87 *mol*/*L*.

Results and Discussion. Experiments have shown that cumene oxidation in the presence of all used extracts kinetic curves of oxygen absorption take place with a distinct induction period, which indicates the presence of AO in used extracts. The typical kinetic curves of cumene oxidation in the absence and in the presence of extracts are illustrated in Fig. 1. The total content of AO ($f \cdot$ [InH]) was determined straightening the experimental data in the coordinates of Eq. (1) (Fig. 2).

In the studied extracts the largest quantity of AO are containing the extracts of the leaves of Evening Primrose ($1.25\cdot10^{-4}$ *mol/L*) and Scarlet Amaranth ($1.15\cdot10^{-4}$ *mol/L*). The results are shown in the Table.

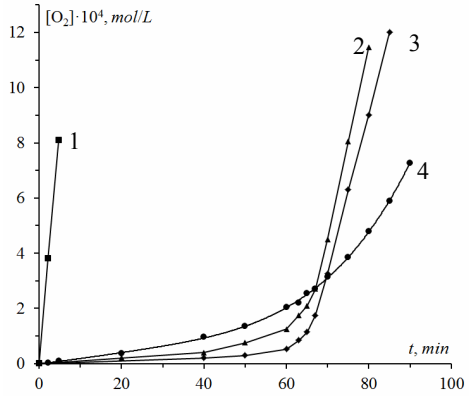

Fig. 1. Kinetic curves of oxygen absorption oxidized cumene in the absence (1) and in the presence of extracts of Savin Juniper needles, 11.52 *mg* (2), Evening Primrose leaves, 3.11 *mg* (3), Pumpkin leaves, 15.75 *mg* (4). $W_i = 1.25\cdot10^{-7}$ *mol/L·s*, T=348.15 *K*.

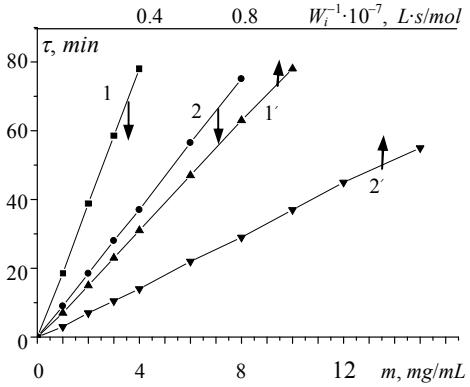

Fig. 2. The dependence of the induction periods of oxygen absorption during the oxidation of cumene (W_i = 1.25 $\cdot10^{-7}$ *mol/L·s*): a) from the extract content from leaves Evening Primrose (1), Sweetleaf (2); b) from the reciprocal of the initiation rate (1′; 2′; $m_{extracts}$=3.11 *mg*). T=348.15*K*.

In order to determine the AOA experimentally measured concentrations of absorbed oxygen during $t<\tau$ were straightened in coordinates of Eq. (2). The correlation between the amount of absorbed oxygen and the parameter $\ln(1- t/\tau)$ is obvious (see Fig. 3).

The tangents of the inclination angles of obtained lines are proportional to k_2/k_7. Taking into account that $k_2 = 4.677\cdot10^6 \exp(-9800 / RT)$ *L/mol·s* for cumene [13], the numerical values of k_7 of investigated extracts (characterizing their AOA) were determined. The results of these calculations are shown in the Table.

The Table shows that the cumene oxidation from the studied extracts, most AOA contain the extracts of Coneflower leaves (at 348.15 *K* $k_7 = 3.21\cdot10^5$ *L/mol·s*), Pumpkin leaves ($2.93\cdot10^5$), Goosefoot herb ($2.7\cdot10^5$), Grecian Silk Vine ($2.59\cdot10^5$) and Alpine Aster ($1.95\cdot10^5$). The data show that the AOA of extracts are the same quality as synthetic-classic antioxidants are. For example, the rate reaction constant with cumene of peroxide radicals for α-naphthol at 333.15 *K* $k_7 = 1.6\cdot10^5$ *L/mol·s*, for hydroquinone $k_7 = 1.2\cdot10^5$, for ionol $k_7 = 2\cdot10^5$, etc. [14].

The comparison of the results (see Table) for AO contents ($f\cdot$ [InH]) and AOA (k_7) in studied extracts shows no association between these parameters, as was expected. This fact once again confirms that AOA does not depend on the concentration of the inhibitor, i.e. does not depend on the content of AO in the reaction mixture. AOA is likely to depend on the structure of AO and $RO_2^{\bullet}$ radicals, as well as on the reaction medium [15].

Experiments have shown that in the presence of the studied extracts, after leaving the induction periods of oxygen absorption on kinetic curves do not tend

to inhibited cumene oxidation (except extracts from the leaves of Sweetleaf) (compare the tangents of the angles of lines on Fig. 1).

One can find similar effects in [15, 16].

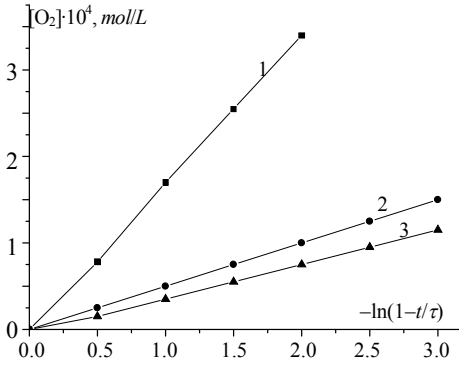

Fig. 3. The dependence of the concentration of oxygen absorbed by the parameter $\ln(1-t/\tau)$ during the induction period in the oxidation of cumene in the presence of extracts from needles of Savin Juniper(1), Evening Primrose leaves (2), Gourd Pumpkin leaves (3).
$W_i = 1.25 \cdot 10^{-7}$ *mol/L·s*, T=348.15 *K*.

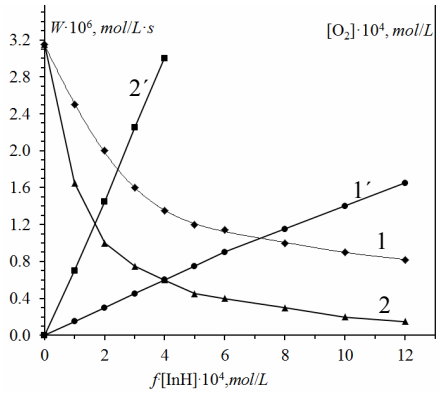

Fig. 4. The dependence of the rate of cumene oxidation after leaving the induction periods the concentration of antioxidants contained in the extracts from the needles of Savin Juniper (1) and Scarlet Amaranth leaves (2) and their straightening (1′; 2′) in the coordination of lev. 3.
$W_i = 1.25 \cdot 10^{-7}$ *mol/L·s*, T = 348.15 *K*.

This is due to the antioxidant properties of oxidation products (Q) of initial AO, presented in the extracts.

The products Q are formed by the reaction:

$$\text{III. } RO_2^{\bullet} + In^{\bullet} \xrightarrow{k_8} Q + \text{product molecules.}$$

Where the products Q differ from the initial AO as their presence on the kinetic curves are not found in induction periods, i.e. their open circuit is carried out as quadratic:

$$\text{IV. } RO_2^{\bullet} + RO_2^{\bullet} \xrightarrow{k_6} \text{product molecules;}$$

so linear

$$\text{V. } RO_2^{\bullet} + Q \xrightarrow{k_{71}} Q^{\bullet} + \text{product molecules;}$$

$$\text{VI. } RO_2^{\bullet} + Q \xrightarrow{k_{81}} \text{product molecules.}$$

In these conditions, the rate of cumene oxidation after leaving the induction period is described by following equation

$$W = k_2[RH]\frac{k_{71}[Q]}{k_6}\left[\left(1+\frac{k_6 W_i}{k_{71}^2[Q]^2}\right)^{1/2} - 1\right]. \quad (3)$$

Taking into account that in the absence of AO cumene oxidation rate (W_0) is

$$W_0 = \frac{k_2}{\sqrt{k_6}}[RH]\sqrt{W_i}, \quad (4)$$

we obtain

$$F = W_0/W - W/W_0 = k_{71} f[Q]/\sqrt{k_6 W_i}, \quad (5)$$

where k_6 and k_{71} are constant rates of reactions (IV) and (V). Here k_{71} characterizes AOA of Q.

As it is seen from Fig. 4, the experimental data are straitened in Eq. (5) coordinates. Assuming that $[Q]$ is equal to the initial concentration of AO in the currant extract, i.e. $[Q]=f\cdot[\mathrm{InH}]$, from the tangents of the inclination angles of (1′) and (2′) value of k_{71} was calculated (here for cumene $k_6=4.74\cdot10^5\exp(-1800/RT)$ was taken into account [13]). The results are given in the Table.

Total content of antioxidants in the ethyl acetate extracts of certain plants and their antioxidant activity (T = 348.15 K)

№	Plant	Stuff	Harvest time	$f\cdot[\mathrm{InH}]$, 10^{-4} *mol/L*	$k_7\cdot10^{-4}$, *L/mol·s*	lg *A*	*E*, *cal/mol*	$k_{71}\cdot10^{-2}$, *L/mol·s*	lg *A*	*E*, *cal/mol*
1	Coneflower	herb	2.08	0.28	32.13	16.96	18240	6.08	10.12	11670
2	Gourd Pumpkin	leaf	7.09	0.30	29.98	9.78	6870	4.60	6.09	5450
3	Goosefoot	herb	12.09	0.53	27.05	6.82	2200	3.84	6.41	6090
4	Evening Primrose	leaf	13.09	1.52	16.12	7.14	3078	3.38	6.67	6600
5	Grecian Silk Vine	leaf	15.09	0.69	25.86	15.11	15450	2.42	5.94	5660
6	Alpine Aster	leaf	15.06	0.65	19.51	11.84	10425	3.25	9.73	11500
7	Scarlet Amaranth	leaf	22.05	1.15	10.15	10.65	8990	2.12	5.75	5450
8	Sweetleaf	leaf	*a*	0.71	9.66	14.05	14420	–	–	–
9	Savin Juniper	needles	25.12	0.41	5.61	12.08	11660	9.71	6.40	5440
10	Pomelo	fruit	*b*	0.31	5.78	11.54	10790	6.26	6.62	6080
11	Field Pea (green)	grain	*b*	0.14	7.64	17.06	19400	8.10	7.61	7490
12	Field Pea (white)	grain	*b*	0.13	10.37	11.06	9620	11.66	9.37	10040

NB: *a* is obtained at the pharmacy; *b* is obtained in the supermarket.

One can see from Table, the highest AOA have the oxidation products of the following extracts: Pea seeds (k_{71} = $11.66\cdot10^2$ *L/mol·s*), Savin Juniper needles (k_{71} = $9.71\cdot10^2$ *L/mol·s*) and Pomelo's fruit (k_{71} = $6.26\cdot10^2$ *L/mol·s*).

In temperature range 328.15–348.15 *K* the temperature dependences of the parameters of k_7 and k_{71} in Arrhenius coordinates are defined (see Table).

It follows that the calculated values of activation energy (*E*) and the pre-exponential factor (*A*) are substantially higher than the same values for the classical oxidation inhibitors in non-polar medium [14].

Abnormally high values of *E* and *A* can be explained by the theory of electrostatic models [17], according to which

$$E = E_0 + \lambda(\varepsilon - 1)/(2\varepsilon + 1), \tag{6}$$

$$A = (RT / N\hbar)\exp(\Delta S^{\neq} / R), \tag{7}$$

$$\Delta S^{\neq} = \Delta S_0^{\neq} + \sigma(\varepsilon - 1)/(2\varepsilon + 1), \tag{8}$$

where *E* is the experimentally measured activation energy, $\Delta S^{\neq}$ is the activation entropy, E_0 and $\Delta S_0^{\neq}$ are their values when the reaction proceeds in a neutral

medium respectively, λ and σ are parameters characterizing the energy and entropy components of the free activation energy respectively, ε is dielectric constant of the reaction medium. From the Eqs. (6), (7) and (8), we obtain the correlation between the pre-exponential factor and energy activation:

$$\lg A = \alpha E + \beta, \tag{9}$$

where $\alpha = \sigma / 2.3R$, $\beta = \lg\left(\frac{RT}{N\hbar}\right) + \frac{\Delta S_0^{\neq}}{2.3R} - \frac{\sigma}{2.3\lambda R} E_0$ at 348.15 K,

$$\beta = 12.86 + 0.22\Delta S_0^{\neq} - 0.22\frac{\sigma}{\lambda} E_0.$$

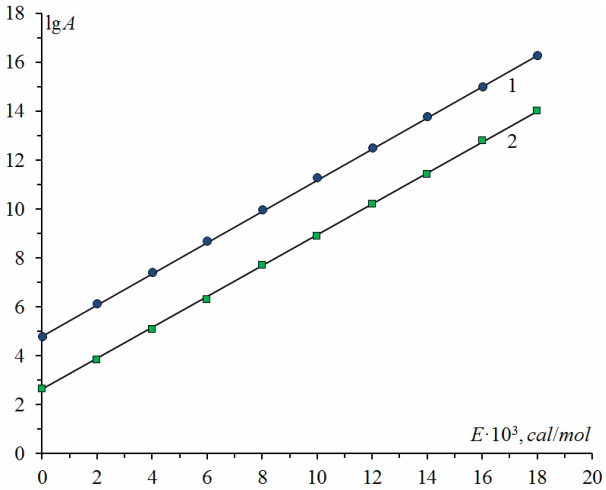

Fig. 5. Dependence lg$A$ on activation energy of the reaction rate constants k_7 (1) and k_{71} (2).

Fig. 5 shows, that for the reaction rate constants k_7 and k_{71} experimentally measured values of pre-exponential factor and the energy activation are straightened in Eq. (9) coordinates. Using the method of least squares were found $\lg A = 4.9 + 0.64E$, $\lg A = 2.6 + 0.64E$ for constants k_7 and k_{71} respectively.

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