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QUANTITATIVE ANTIOXIDANT ACTIVITY OF THE ETHYLACETATE EXTRACT OF *Larix sibirica* BARK AND ITS INDIVIDUAL COMPONENTS

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The antioxidant [AO] properties of the ethylacetate extract of Larix sibirica *bark and the flavonoids quercetin and dihydroquercetin found in it were studied using a model radical-chain oxidation of propan-2-ol in the kinetic regime. The quantitative characteristics of their AO activity were determined as effective rate constants fk_{In}. It was found that dihydroquercetin had the highest AO activity among the studied natural compounds.*

Key words: *Larix sibirica* Ledeb., flavonoids, antioxidants, radical-chain reactions, initiated oxidation, inhibition rate constant.

The complex of monomeric phenolic compounds from *Larix sibirica* Ledeb. bark may have high biological activity and low toxicity and may be used to fabricate capillary-protective phytopreparations [1, 2].

The complex of monomeric polyphenolic compounds was isolated from Siberian larch bark by the literature method [1, 2]. This extract is known to contain *p*-hydroxybenzoic, protocatechoic, vanillic, syringic, *p*-coumaric (*cis*- and *trans*-forms), ferulic (*cis*- and *trans*-forms), and caffeic acids [1]. The identified flavonoid compounds include dihydroquercetin, dihydrokaempferol, the five flavonols quercetin, kaempferol, morin, myricetin, and isorhamnetin, and the flavonone naringenin. Furthermore, this fraction contains also four catechinoic compounds and an anthocyanidine dye [1].

The goal of the present work was to investigate the antioxidant activity (AOA) of Siberian larch extract and the pure compounds quercetin (Q) and dihydroquercetin (DQ) using initiated oxidation of propan-2-ol as a model reaction. These investigations are also important because the antioxidant (AO) properties of the compounds are just one of the aspects of their biological activity [3]. Initiated oxidation of propan-2-ol occurs by a radical—chain mechanism in the kinetic regime. According to this, chain termination under the experimental conditions occurs via reaction of hydroxyperoxyl radicals with each other [4]. Adding to the reaction mixture compounds that possess AO properties reduces the oxidation rate of the model substrate by terminating the chain at the inhibiting impurity [reaction (3)] [5]:

$$(CH_3)_2CH(OH) \xrightarrow{\dot{r}, O_2} (CH_3)_2C(OH)OO^{\cdot} \quad (1)$$

$$(CH_3)_2C(OH)OO^{\cdot} + (CH_3)_2C(OH)H \longrightarrow (CH_3)_2C(OH)OOH + (CH_3)_2\dot{C}(OH) \quad (2)$$

$$FLOH + (CH_3)_2C(OH)OO^{\cdot} \longrightarrow FLO^{\cdot} + (CH_3)_2C(OH)OOH \quad (3)$$

$$FLO^{\cdot} + (CH_3)_2C(OH)OO^{\cdot} \longrightarrow FLOH + (CH_3)_2C{=}O + O_2 \quad (4)$$

$$FLO^{\cdot} + (CH_3)_2C(OH)OO^{\cdot} \longrightarrow \text{Products}, \quad (5)$$

where FLOH are the flavonoids Q and DQ and $FLO^{\cdot}$ are the products of their reaction with hydroxyperoxyl radicals of propan-2-ol.

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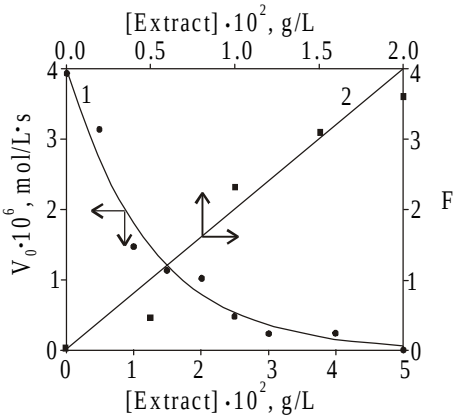

Fig. 1

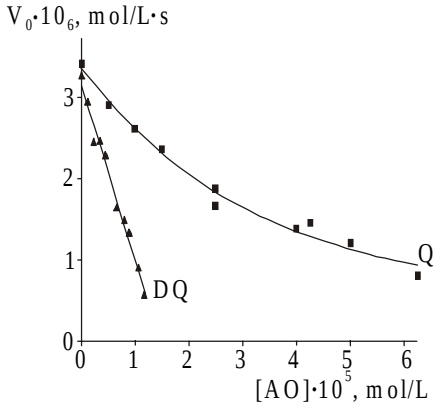

Fig. 2

Fig. 1. Rate of oxygen absorption as a function of initial concentration of added larch extract (1) and its anamorphosis (2) in coordinates of Eq. (1); $V_i = 1 \cdot 10^{-7}$ mol/L·s, T = 348 K.

Fig. 2. Initial oxidation rate of propan-2-ol as a function of quercetin and dihydroquercetin concentration; $V_i = 1 \cdot 10^{-7}$ mol/L·s, T = 348 K.

Thus, the presence of an AO should reduce the oxidation rate of propan-2-ol. This was observed experimentally.

Hydroxyperoxyl radicals perform two functions. They can act as an oxidant [reaction (2)] or as a reductant [reaction (4)] [4]. Reaction of the radical inhibitor FLO$^{\cdot}$ with hydroxyperoxyl radicals of propan-2-ol by reaction (4) regenerates the starting AO. This increases the effectiveness of its AO action.

The quantitative parameters of the AOA are the rate constant for chain termination of oxidation fk_{ln}, where f is the radical capacity of the AO that indicates the number of hydroxyperoxyl radicals consumed per single AO molecule during chain termination [5].

The AOA of samples was studied for [AO] = $(0.5\text{-}5.0) \cdot 10^{-2}$ g/L for the extract, $(0.50\text{-}6.25) \cdot 10^{-5}$ mol/L for Q, and $(1.12\text{-}9.00) \cdot 10^{-6}$ mol/L for DQ, where [AO] is the AO concentration. The experiments showed that the complex of monomeric phenolic compounds and flavonoids Q and DQ isolated from it had high AOA (Figs. 1 and 2). Thus, adding Q and DQ to the model system produced a regular decrease in the oxidation rate of propan-2-ol by 4.2 and 6.00 times for Q and DQ, respectively. Equation (1) was used to determine fk_{ln} [5]

$$F = V_0^0/V_0 - V_0/V_0^0 = fk_{In}[AO]/(2k_6Vi)^{1/2}, \qquad (1)$$

where V_0^0 and V_0 are the initial rates of oxygen absorption for propan-2-ol oxidation in the absence and presence of AO, respectively, [AO] is the concentration of added AO, and k_{ln} and $2k_6$ are the rate constants for oxidation chain termination at AO and second-order chain termination at substrate hydroxyperoxyl radicals, respectively [5]. The inhibition parameter F is a relative value. Thus, the rate of inhibited propan-2-ol oxidation in the presence of *L. sibirica* bark extract was expressed in mol/L·s.

It was found that Eq. (1) is obeyed satisfactorily for all studied compounds (correlation coefficient > 0.95, Figs. 1 and 2). The linear relationship between the inhibition parameter calculated using Eq. (1) and the concentrations of the tested AO in the concentration range $(0.0\text{-}2.0) \cdot 10^{-2}$ g/L for the extract, $(0.5\text{-}5.0) \cdot 10^{-5}$ mol/L for Q, and $(1.12\text{-}9.00) \cdot 10^{-6}$ mol/L for DQ indicates that second-order chain termination of inhibited propan-2-ol oxidation dominated in these concentration ranges.

The quantity $fk_{ln}/(2k_6 \cdot V_i)^{1/2}$ was determined from the slopes of these plots for each sample (Fig. 3). Based on the experimental results, we found the effective inhibition rate constants fk_{ln}. These quantities were calculated using the published value $2k_6 = 2 \cdot 10^8$ L/mol·s [6]. Since the concentration of the Siberian larch bark extract was measured in g/L, a recalculated value of $3.33 \cdot 10^6$ L/g·s for $2k_6$ and an experimental value $V_i = 1.64 \cdot 10^{-5}$ g/L·s were used to calculate its quantitative parameters.

TABLE 1. Kinetic Parameters of Investigated Compounds

Compound	$fk_{ln}\cdot10^{-5}$, L/mol·s	$fk_{ln}\cdot10^{-2}$, L/g·s	IE
Quercetin	2.5±0.2	8.3±0.7	2.5
Dihydroquercetin	11±1	36.0±3.3	11
Larch extract	-	14.2±3.6	3.15
Ionol	1.0±0.2	4.5±0.9	1.0

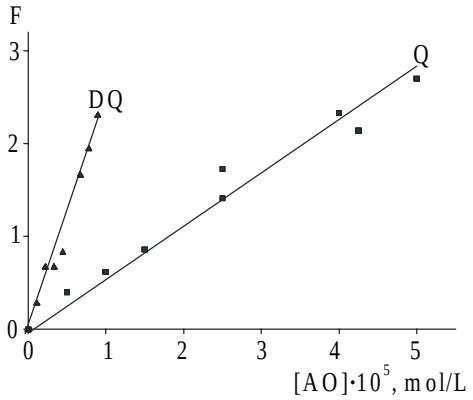

Fig. 3. Effectiveness of inhibition as a fucntion of quercetin (Q) and dihydroquercetin (DQ) concentration according to Eq. (1); $V_i = 1\cdot10^{-7}$ mol/L·s, T = 348 K.

Table 1 gives effective inhibition rate constants for Q and DQ. The ionol equivalents calculated from Eq. (2) are also given in Table 1 in order to compare the efficiencies of AOA of these compounds.

$$IE = fk_{ln}/fk_{ionol}. \quad (2)$$

The results led to the conclusion that the AO efficiency of Siberian larch bark extract is more than three times greater than the known AO ionol. The high level of AOA of the extract is explained by the presence in it of representatives from practically all flavonoid classes, starting with the flavanone naringenin to biflavonoids, proanthocyanidines, and condensed tannins. The type of hydroxylation of ring B assigns the compounds to two principal groups: *p*-hydroxyphenyl (monosubstituted) and pyrocatechin (disubstituted), with the former dominating. It is known that pure representatives of these flavonoid classes are efficient AOs of peroxide oxidation of cell-membrane lipids in animals and humans [7-16].

Table 1 also shows that the AO efficiency of the extract is about 2.5 times less than that of DQ, which exhibits the greatest AOA in the model system used by us. This suggests that antagonism is probably observed upon reaction of the pure components in the extracted mixture. A more detailed study of the nature of these phenomena will be the subject of further research.

EXPERIMENTAL

The reactivity of Q, DQ, and *L. sibirica* Ledeb. extract as AO agents was studied at 348 K and initiation rate $V_i = 1\cdot10^{-7}$ mol/L·s in the kinetic oxidation regime [4, 5]. Azodiisobutyronitrile (AIBN) was used as the initiator.

The efficiency of the AOA of the studied compounds was evaluted from the degree of reduction of the initial rate of atmospheric oxygen absorption upon oxidation of the model substrate in their presence. The rate of oxygen absorption was determined by a highly sensitive universal differential manometer apparatus, the construction of which has been described [17]. Propan-2-ol was purified beforehand as before [18].

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