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## ANTIOXIDATIVE ACTIVITY OF *RUBUS IDAEUS* (L.) ONE-YEAR SHOOTS

**D.K. Gulyaev**, Candidate of Pharmaceutical Sciences, Assistant Professor of the Department of Pharmacognosy with a course in Botany, Perm State Pharmaceutical Academy, Perm, Russia

**V.D. Belonogova**, Doctor of Pharmacy, Professor, Head of the Department of Pharmacognosy with a course in Botany, Perm State Pharmaceutical Academy, Perm, Russia

**D.O. Bokov**, Candidate of Pharmaceutical Sciences, Associate Professor of the Department of Pharmaceutical Science of the Institute of Pharmacy named after A.P. Nelyubin, I.M. Sechenov First Moscow State Medical University, Moscow, Russia, Research Scientist at the Laboratory of Food Chemistry, Federal Research Center of Nutrition and Biotechnology, Moscow, Russia

**V.V. Bessonov**, Doctor of Biological Sciences, Head of the Laboratory of Food Chemistry, Federal Research Center of Nutrition and Biotechnology, Moscow, Russia

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The purpose is to study the phenolic compounds and antioxidant activity of the dry aqueous extract of the *Rubus idaeus* (L.) one-year shoots. The objects of the study were samples of one-year shoots of red raspberry harvested on the territory of the Ilyinsky district of the Perm Region in the herbaceous-spruce forest in June 2019. Young shoots up to 30 cm long were cut and dried with a shade drying method.

The analysis of phenolic compounds was carried out with an Agilent 1100 Series HPLC liquid chromatograph. To determine the antioxidant activity, a reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used. As a result of a chromatographic study, 12 substances of a phenolic nature were identified in the dry aqueous extract of red raspberry one-year shoots. The dry extract of raspberry shoots contains in the greatest amount – ellagic, benzoic, vanillic acids, vanillin and the flavonoid hyperoside. Dry aqueous extract of raspberry shoots shows pronounced antioxidant activity ( $IC_{50}$  – 43.86  $\mu\text{g/ml}$ ). This indicator exceeds the activity of the of the sweet-brier fruits infusion in terms of dry residue ( $IC_{50}$  – 76.75  $\mu\text{g/ml}$ ), but is inferior in terms

of the antioxidant effect of the ascorbic acid substance ( $IC_{50}$  – 9.46  $\mu\text{g/ml}$ ). Dry aqueous extract of raspberry one-year shoots can be recommended for further studies of antioxidant and other types of pharmacological activity.

**Keywords:** red raspberry, one-year shoots, dry aqueous extract, phenolic compounds, antioxidant activity

The etiology of many cardiovascular and oncological diseases is associated with disorders that occur as a result of the exposure to body cells of free radicals, which are ions or molecules that have an unpaired electron on the valence orbital (peroxyl radical, superoxide anion radical). Most of the body's structures (nucleic acids, proteins, fats and carbohydrates) are potential targets for free radical damage [1].

Many researchers point to the dominant role of phenolic compounds in the development of the antioxidant activity of medicinal herbs [2–5]. Some studies of *Rubus* species show a correlation between the content of phenolic compounds and the level of antioxidant activity [6].

The official medicinal plant raw materials of red raspberries are fruits, but the leaves accumulate a greater amount of phenolic compounds, including polyphenols, which allow you to show antioxidant activity higher than that of fruits [7].

The N-butanol fraction of the ethanol extract of the shoots of *Rubus parvifolius* L. (small-leaved raspberry) has a pronounced hepatoprotective effect against carbon tetrachloride (CCl<sub>4</sub>) – induced liver damage, which is partly due to the strong antioxidant ability of the extract [8].

Among the most interesting classes of substances which are responsible for the development of antioxidant action in the shoots and leaves of species of the *Rubus* genus, there are ellagic acid derivatives and flavonoids.

Ellagotannins of *Rubus* species also have high antioxidant activity. The structures of ellagotannins are characterized by the presence of several hydroxyl groups in the ortho position, which exhibit the ability to give off a hydrogen atom [6].

**The Purpose of work** – research of phenolic compounds and antioxidant activity of dry aqueous extract of *Rubus idaeus* (L.) one-year shoots

## MATERIALS AND METHODS

The objects of the study were samples of young green one-year shoots of red raspberry – *Rubus idaeus* (L.). Raspberry shoots were harvested on the territory of the Ilyinsky district of the Perm Territory in spruce forest in June 2019. Young one-year shoots with a length of up to 30 centimeters were cut and dried with a shade drying method.

The extract of dry aqueous shoots of red raspberry was obtained by the following method: a sample weight of air-dried raw materials was placed in a round-bottomed flask, overwatered with water purified in a ratio of 1:30, and extracted with constant stirring for an hour and a half at

temperature of 80°C. The solvent cake was separated by filtration and the extract was evaporated with a rotary evaporator.

Three times the amount of 95% ethyl alcohol was added to the evaporated residue, as a result of which the water-soluble polysaccharide complex precipitated. Polysaccharides were separated by filtration through a paper filter, washed out with 80% ethyl alcohol. The extract obtained after separation of polysaccharides was evaporated using a rotary evaporator. The evaporated residue was placed in a drying cabinet and dried at a temperature of 55°C.

The analysis of phenolic compounds was carried out using an Agilent 1100 Series HPLC liquid chromatograph complete with a two-solvent feed and degassing system, a diode-matrix detector, a column thermostat, and an automatic sample input device (autosampler). Software – Agilent ChemStation Rev. A. 09. 03, a column – Atlantis dc18, 100A, 5 microns, 4.6×250 mm. A 0.1% formic acid solution, methanol/acetonitrile (25:75), was used as a mobile phase. The elution mode is gradient, the column temperature is 35°C, the flow rate of the mobile phase is 0.8 ml/min and the volume of the injected sample is 20 µl.

To determine the antioxidant activity, a reaction with a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used [9]. To 1 ml of the test solution, 3 ml of DPPH solution in 95% ethyl alcohol with a concentration of 5 mg/100 ml was added. Next, the antioxidant activity and radical uptake were calculated using the formula:

$$\text{AOA (\%)} = (A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}} \times 100.$$

We also determined the value of IC<sub>50</sub> – the concentration of the substance that can bind half the concentration of the DPPH radical, determined by the inhibition curve obtained when plotting the inhibition graphs as a percentage of the concentration of the substance. As a reference substance, ascorbic acid and a decoction of rosehip fruits were used, since rosehip is a vitamin raw

material and accumulates ascorbic acid and other antioxidants. A decoction of rosehip fruits was prepared in accordance with GPM 1.4.1.0018.15. "Infusions and decoctions" [10].

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## RESULTS AND DISCUSSIONS

Many phenolic compounds take an active part in redox processes and promote the binding of reactive oxygen intermediate. Therefore, the content of phenolic compounds in plant extracts is an important indicator that characterizes the possibility of antioxidant and anti-radical effects.

The results of determining the content of phenolic compounds in the extract of raspberry shoots are presented in Table 1.

Table 1 shows that among the identified substances of a phenolic nature, the ellagic acid is contained in the largest amount in the dry extract of raspberry shoots. The content of benzoic acid, vanillic acid, vanillin and hyperoside is also high.

Some studies suggest that the active metabolite of ellagic acid, urolitin A, can cause neuroprotective effects and protect neurons from oxidative damage [11].

The antioxidant effect of ellagic acid and its metabolites can prevent some cardiovascular diseases associated with oxidative stress by affecting the molecular mechanisms of the vascular bed [12].

In one of the studies in an experiment in mice with type 2 diabetes after administration of vanillin acid for 8 weeks at a dose of 50 mg/kg, a significant decrease in fasting blood glucose

Table 1

### CONTENT OF PHENOLIC COMPOUNDS IN THE EXTRACT OF RASPBERRY SHOOTS

Substance	Wave-length (nm), $\lambda$	Retention time (min), RT	Content, %
Vanillic acid	254	25.0	0.353
Ellagic acid	254	30.0	0.669
Benzoic acid	254	36.3	0.399
Gallic acid	280	13.3	0.030
Vanillin	280	30.0	0.343
Caffeic acid	300	25.8	0.193
P-coumaric acid	300	30.8	0.042
Ferulic acid	300	32.6	0.092
Salicylic acid	300	38.1	0.074
Hyperoside	350	31.4	0.221
Quercetine	350	43.8	0.054
Kaempferol	350	48.5	0.023

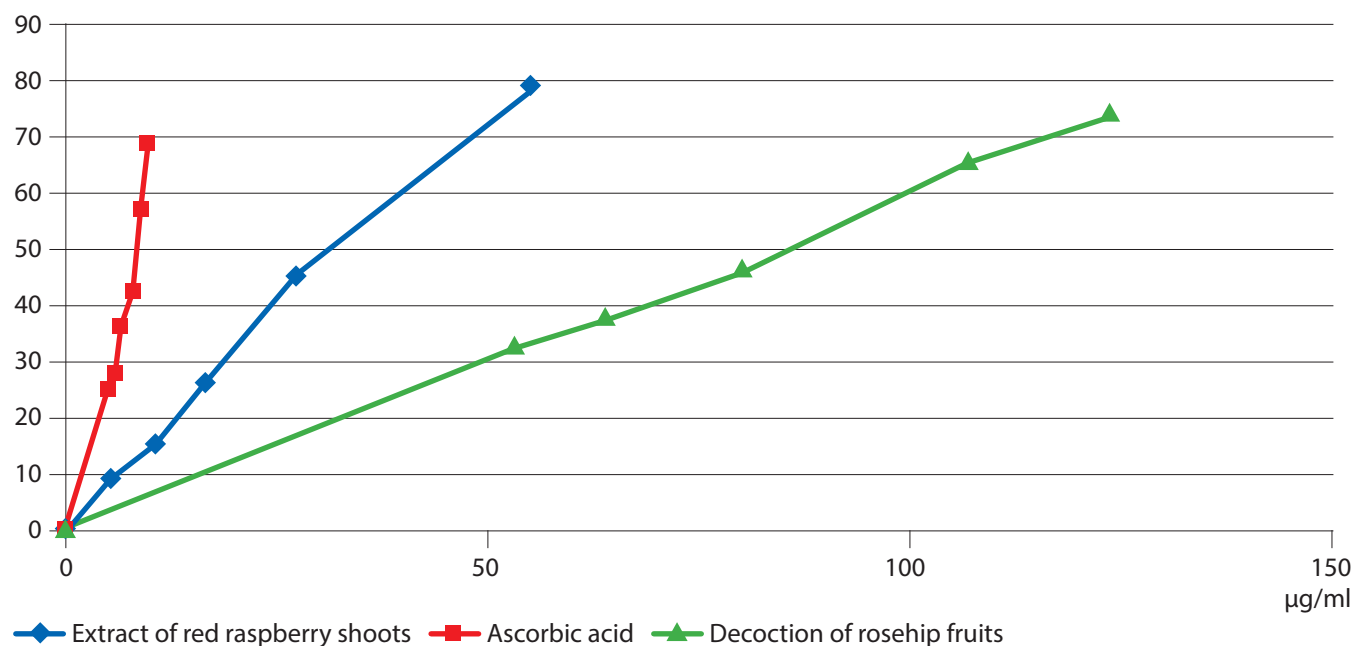


FIG. 1. Antioxidant activity (%) of dry water extract of first-year raspberry shoots

and blood pressure was observed in comparison with the control group. Also, in animals treated with vanillic acid, markers of lipid peroxidation were reduced, which suggests that vanillic acid helps to combat the oxidative stress [13]. Vanillic acid isolated from the leaves of *Rubus chingii Hu* showed pronounced antioxidant properties [14].

It was interesting to study the antioxidant activity of an aqueous dry extract of first-year raspberry shoots. The results of the determination are shown in Fig. 1 and in Table 2.

Figure 1 shows the dependence of antioxidant activity on the concentration of the studied

substances. For red raspberry shoot extract in the concentration range from 5 to 55 µg/ml, the monotonic and spur increase in activity from 10 to 80% is observed. For the reference substance, which was ascorbic acid, in the concentration range from 5 to 11 µg/ml, a spur increase in activity from 0 to 70% is characteristic.

As a result of the study, it was found that the dry water extract of *Rubus idaeus* shoots is able to exhibit pronounced antioxidant activity (Table 2). To correlate the results with the action of known antioxidants, we simultaneously analyzed the substance of ascorbic acid. The IC<sub>50</sub> for the ascorbic acid substance was found to be

Table 2

**ANTIOXIDANT ACTIVITY OF DRY WATER EXTRACT OF THE RED RASPBERRY (*RUBUS IDAEUS L.*) ONE-YEAR SHOOTS (IC<sub>50</sub>)**

Sample	Antioxidant activity, IC <sub>50</sub> , µg/ml
Dry water extract of the red raspberry one-year shoots	43.86±10.06*
Ascorbic acid	9.46±0.93**
Decoction of rosehip fruit	76.75±14.62

\* In relation to raspberry shoot extract (t-test) – Student t-test p<0,05

\*\* In relation to the decoction of rosehip fruits (t-test) – Student t-test p<0,05

9.46 µg/ml. Thus, 1 gram of dry water extract of *Rubus idaeus* shoots corresponds to 0.14 g/ equivalent of ascorbic acid. The infusion of rosehip fruits in terms of dry residue shows antioxidant activity almost 2.5 times lower than the studied extract of red raspberry shoots

## CONCLUSIONS

As a result of the study, it was found that the dry water extract of *Rubus idaeus* shoots is able to exhibit pronounced antioxidant activity (Table 4). To correlate the results with the action of known antioxidants, we simultaneously analyzed the substance of ascorbic acid. The IC<sub>50</sub> for the ascorbic acid substance was found to be 9.46 mcg/ml. Thus, 1 gram of dry water extract of *Rubus idaeus* shoots corresponds to 0.14 g/ equivalent of ascorbic acid. The infusion of rosehip fruits in terms of dry residue shows antioxidant activity almost 2.5 times lower than the studied extract of raspberry shoots

As a result of the study, 12 substances of a phenolic nature were identified. The dry extract of raspberry shoots contains ellagic acid in the largest amount. The content of benzoic acid, vanillic acid, vanillin and hyperoside is also high.

Dry water extract of red raspberry one-year shoots shows a pronounced antioxidant activity, exceeding the activity of the infusion of rosehip fruits, but several times inferior in activity to the reference substance, which was ascorbic acid. Thus, the one-year shoots of red raspberry can be considered as a source of antioxidants and promising for further study.

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