Study of the antioxidant activity of rowan extracts (Sorbus aucuparia) by biotesting method

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Abstract. Special importance in modern unfavorable environmental conditions is given to nutrition, which should ensure the weakening of the negative effect of harmful eco-factors on the human body, contribute to increasing its protective and adaptive capabilities, and, as a result, reduce the risk of developing various pathologies. At the same time, it should be taken into account that the diet of a modern person should be rich in biologically active components that can exhibit antioxidant properties. Recently, scientists are increasingly considering plant extracts as sources of antioxidants. Among them, a special role is assigned to Sorbus aucuparia, whose antioxidant activity is described as comparable, or even higher than other fruits. The article presents data on the study of the antioxidant activity of water extracts of mountain ash by biotesting. The antioxidant activity was evaluated using a biological model – Paramecium caudatum, determining the stress resistance of infusoria to hydrogen peroxide. The study revealed an increase in the stress resistance of Paramecium caudatum infusoria when adding extracts of mountain ash to the nutrient mixture compared to the control.

1. Introduction
Cardiovascular, autoimmune, neurodegenerative diseases, atherosclerosis, cancer are the result of spontaneous oxidative stress, the causes of which are caused by unfavorable conditions of the development of modern society, associated with increasing rates of negative anthropogenic impact on the environment, high tension, psychoemotional loads[1, 2].

As a result, there is a need to increase the resistance of the body, which can be achieved by enriching the human diet at the expense of natural biocorrectors. The composition of the latter includes a complex of biologically active substances that can normalize the state of the body. First of all, this applies to herbal preparations characterized by pronounced antioxidant activity, and the absence of side effects compared to synthetic biologically active supplement.

Within the framework of import substitution, the primary task of the domestic agro-industrial complex today is to expand the range of products based on domestic phytomaterials, among which it is possible to distinguish the fruits of mountain ash (Sorbus aucuparia), growing on the territory of the Republic of Tatarstan. The choice of phytomaterials is due to its chemical composition, which includes a valuable natural complex of biologically active substances.
However, the well-known instrumental methods that determine the above indicators are characterized by a fairly high duration, the need for expensive reagents and, at the same time, insufficient information content, given the complexity of the composition of vegetable raw materials. In this regard, to assess the quality of agro-industrial complex, preference has recently been given to biological methods of express diagnostics based on the use of biotesting, which is characterized by high sensitivity, reproducibility, integrality, and ease of analysis execution.

In this regard, the aim of the work was to evaluate the antioxidant activity of extracts of mountain ash fruits.

2. Materials and methods of research
The objects of the study were water extracts of the fruits of mountain ash (*Sorbus aucuparia L.*) obtained: the ratio of raw materials to extractant 1:6; temperature 65°C; extraction time 1.5 h; mixing speed 500 rpm, in accordance with the recommendations given in works [3, 4].

The subject of the study was the antioxidant activity of rowan extracts, which was determined on the basis of the stress resistance of *Paramecium caudatum* equilateral infusoria.

2.1. Preparation of Paramecium caudatum infusoria culture for biotesting.
A 3-day culture of *Paramecium caudatum* infusoria was used in this work. Their cultivation was carried out at room temperature in test tubes on mineral medium Lozin-Lozinski [5]. The substrate for the infusoria during cultivation was a suspension of the baking yeast *Saccharomyces cerevisiae*. To prepare the nutrient mixture, 15-20 granules of dry yeast were dissolved in 10 cm³ of the medium. Feeding of infusoria was carried out daily by 1-2 drops of yeast suspension per test tube with a volume of 10 cm³.

An indicator of a good state of culture is the formation of a "ring" by paramecia below the meniscus of the test tube [6].

2.2. Determining the stimulation and inhibitory effects of extracts on the growth and reproduction of the test object.
The nutrient mixtures, including the studied 1% phytoextract on an absolutely dry substance, were prepared in sterilized penicillin bubbles, into which 6 cm³ of the autoclaved Lozin – Lozinsky medium, 40 µl of yeast suspension (as the main substrate) and an extract from 5 to 500 µl were added. The determination of dry matter in the extracts of mountain ash fruits was carried out by the standard method [7]. The nutrient mixtures were stored in the refrigerator.

One infusoria of a 3-day culture was placed in the cavities of the microaquarium, trying to minimize the amount of liquid entering the cavity from the test tube together with the infusoria. Then the cavities were filled with the analyzed nutrient mixture in the amount of 300 ml.

After that, the microaquarium was covered with glass plates, and it was placed in a tray, the bottom of which was covered with moistened filter paper. After 24 hours, the increase in the number of infusoria was observed in each well using an MBS -9 microscope, their mobility and changes in morphological properties, with the subsequent repetition of the above operations with the offspring of the 1st and 2nd generations of infusoria when using a nutrient mixture of the same composition. The results were evaluated after 24 hours by the presence of live (mobile) infusoria.

The experiment was carried out for three days because the adaptive capabilities of infusoria can manifest themselves after a certain time or only in the offspring.

For control, a nutrient mixture was added without phytoextract.

2.3. Determination of the stress resistance of infusoria.
A 3-day culture of infusoria was transplanted into test tubes with Lozin-Lozinsky medium in the amount of 20-40 individuals (600-800 µl). 500 µl of the studied nutrient solution was added to the test tube every day and the infusoria were cultured for 3 days. The stress resistance of the infusoria to hydrogen peroxide was checked for 3 days [8]. In this case, a 1.5% solution of hydrogen peroxide was used, the concentration of which was determined experimentally [5].
To study the stress resistance of infusoria, a microaquarium with cavities was used, which was placed on the microscope slide table. Observation of the paramecia was carried out using a microscope «MBS-9» at a magnification of ×14. 4-5 individuals were placed in the cavity using a capillary pipette. In this case, the amount of culture fluid in the well should not exceed 0.02 cm³.

300 µl of a stressor of the appropriate concentration was poured into the well with infusoria using a dispenser with a removable sterile tip and the time of complete immobilization of the paramecia was recorded. The experiment was carried out in a 3-fold repetition.

### 3. Results and discussion.

Initially, a range of concentrations of extracts that did not inhibit the growth and reproduction of the test object was selected, since plant extracts contain substances such as phytoalexins, terpenoids, essential oils [9].

For a preliminary assessment of the effect of rowan extracts on the growth of the test culture, a range of 50-500 µl with a step of 50 was selected. This matched to the following concentrations of extracted substances from the fruits of mountain ash in the nutrient mixture: from 80 µg/ml to 800 µg/ml. The results obtained are shown in Table 1.

Based on the obtained results, a range of phytoextracts concentrations from 0-80 micrograms/ml was selected for subsequent researches. According to the results obtained, there is no need of adding infusoria in the cultivation medium in amounts of more than 50 µl, since its increase inhibits the growth and reproduction of the culture. Since antioxidants are substances that show an effect in micro quantities, clarifying experiments were carried out, providing for varying the volume of the extract from 0-50 µl. The results are presented in Table 2.

| Table 1. The effect of rowan extracts on the growth of infusoria. |
|---------------------------------------------------------------|
| **Experiment 1**                                              |
| Extract concentration, µg/ml                                  |
| days  | control | 80 | 160 | 240 | 320 | 400 | 480 | 560 | 640 | 720 | 800 |
| day 1  | 2\,2\,1 | 2\,1\,1 | 2\,2\,2 | 2\,3\,1 | 1\,- | - | - | - | - | - | - |
| day 2  | 2\,3\,2 | 3\,2\,2 | 2\,2\,2 | 3\,3\,3 | 1\,- | - | - | - | - | - | - |
| day 3  | 3\,4\,2 | 3\,5 | 2\,2\,2 | 3\,2\,2 | \,1\,1 | - | - | - | - | - | - |
| **Experiment 2**                                             |
| Extract concentration, µg/ml                                  |
| days  | control | 80 | 160 | 240 | 320 | 400 | 480 | 560 | 640 | 720 | 800 |
| day 1  | 1\,1\,1 | 2\,1\,2 | 1\,1\,1 | 1\,- | - | - | - | - | - | - | - |
| day 2  | 1\,3\,2 | 2\,3\,2 | 2\,2 | - | - | - | - | - | - | - | - |
| day 3  | 2\,2\,1 | 3\,1\,2 | 1\,- | 1/1/2 | - | - | - | - | - | - | - |
| **Experiment 3**                                             |
| Extract concentration, µg/ml                                  |
| days  | control | 80 | 160 | 240 | 320 | 400 | 480 | 560 | 640 | 720 | 800 |
| day 1  | 2\,3\,2 | 2\,2\,3 | - | - | - | - | - | - | - | - | - |
mountain ash in the above concentrations to the medium of their cultivation (Figure 1). The obtained results indicate that the studied range of phytoextract concentrations does not inhibit the growth of the culture. However there was no pronounced growth-stimulating effect. Which can be explained as follows: antioxidants, performing a protective function, are often not a substrate for biota.

The next step in the work was to check the stress resistance of infusoria by adding an extract of mountain ash in the above concentrations to the medium of their cultivation (Figure 1).

Table 2. The effect of rowan extracts on the growth of infusoria.

| Experiment 1 | Days | Control | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 |
|--------------|------|---------|---|----|----|----|----|----|----|----|----|----|
| Days | | | | | | | | | | | | |
| day 1 | | | | | | | | | | | | |
| day 2 | | | | | | | | | | | | |
| day 3 | | | | | | | | | | | | |
| day 4 | | | | | | | | | | | | |

| Extract concentration, µg/ml | Days | Control | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 |
|------------------------------|------|---------|---|----|----|----|----|----|----|----|----|----|
| day 1 | | | | | | | | | | | | |
| day 2 | | | | | | | | | | | | |
| day 3 | | | | | | | | | | | | |
| day 4 | | | | | | | | | | | | |

| Experiment 2 | Days | Control | 8 | 16 | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 |
|--------------|------|---------|---|----|----|----|----|----|----|----|----|----|
| Days | | | | | | | | | | | | |
| day 1 | | | | | | | | | | | | |
| day 2 | | | | | | | | | | | | |
| day 3 | | | | | | | | | | | | |
| day 4 | | | | | | | | | | | | |

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|------------------------------|------|---------|---|----|----|----|----|----|----|----|----|----|
| day 1 | | | | | | | | | | | | |
| day 2 | | | | | | | | | | | | |
| day 3 | | | | | | | | | | | | |
| day 4 | | | | | | | | | | | | |

The obtained results indicate that the studied range of phytoextract concentrations does not inhibit the growth of the culture. However there was no pronounced growth-stimulating effect. Which can be explained as follows: antioxidants, performing a protective function, are often not a substrate for biota.

The next step in the work was to check the stress resistance of infusoria by adding an extract of mountain ash in the above concentrations to the medium of their cultivation (Figure 1).

![Figure 1](image-url) Figure 1. Results of the stress resistance study for the first (A); second (B); third (C) experiment.
As can be seen from the graphic material, the addition of phytoextract of mountain ash extracts to the cultivation medium of the test object leads to an increase in the stress resistance of *Paramecium caudatum* infusoria by more than 2 times, when the extract is applied in a volume of more than 20 µl, which matches to a concentration of 32 µg/ml (a nutrient medium without the addition of phytoextract was used as a control). This is due to the content of the richest complex of biologically active substances (BAS) in the fruits of mountain ash, which exhibit antioxidant properties. First of all, these are flavonoids, and the increase in the stress resistance of the biotest is explained by their ability to form chelated compounds with metals. The binding of iron or copper ions by flavonoids can significantly reduce the rate of free radical processes. Flavonoids are also able to inhibit many enzymes (xanthine oxidase, protein kinase) and exhibit a radical-trapping ability to superoxide anion radicals [10].

It is important to note that vitamin E and C are able to inhibit the processes of lipid peroxidation. The main antioxidant function of tocopherols is the termination of the process of lipid peroxidation at the stage of continuation of the chain reaction. Vitamin C acts as a powerful regenerating agent. It is able to restore the oxidized radical form of α-tocopherol, thus regenerating and prolonging the life cycle of this antioxidant in the lipid phase and contributing to the removal of radicals from the lipid to the aqueous phase.

With a decrease in the concentration of the extract in the nutrient medium, a significant increase in stress resistance was not observed in most experiments.

4. Conclusion
The optimal conditions for conducting an experiment to determine the stress resistance of *Paramecium caudatum* infusoria when introducing extracts of mountain ash into their cultivation medium were selected. The required volume of the applied extract matches to the range of 5-50 µl.

An increase in the stress resistance of the test object was observed when phytoextract was added to the nutrient mixture at a concentration of more than 32 µg/ml. Compared with the control, the stress resistance of *Paramecium caudatum* infusoria in relation to hydrogen peroxide, which mainly has a destructive effect on membrane lipids, increases by more than 2 times.

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