

Research on the antioxidant activity and plant growth-promoting effect of extracts from *Fagopyrum esculentum* grown under the conditions of Primorsky kray, Russia

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Abstract

Currently, a promising direction for the development of organic agriculture is research on the potential and action spectrum of biologically active compounds produced by vascular plants and the improvement of technologies for their application in production. Buckwheat (*Fagopyrum esculentum* Moench) is a plant species with an exceptional ability to produce secondary metabolites, including flavonoids. It was discovered that *F. esculentum* plants, especially those with red and red-green stems, were a promising source of flavonoid compounds (up to 3%). Buckwheat extracts had high antioxidant activity, which varied from 19.8–99.4% depending on their concentration, and positively influenced root formation in *F. esculentum* plants. The strongest growth-promoting effect on plant roots was produced by 0.1–1.0% extracts from buckwheat variety Bashkirskaya krasnostebel'naya and local varieties Izumrud and Pri 7 with red and red-green stems. Treating buckwheat plants with the extracts from *F. esculentum* under the conditions of Primorsky kray (Russia) at the stages of bud formation increased the accumulation rate of flavonoids in their fruits up to 0.22%. The extracts from *F. esculentum* might be used in organic agriculture as the natural sources of biologically active compounds for promoting the growth of cultivated plants.

Introduction

Great interest has been generated by studies on the biologically active compounds (BACs) that can affect the growth and development of plants and increase the productivity and quality of crops. BACs are extremely diverse in their chemical nature. The main groups are phenolic derivatives (flavonoids, quinones, and phenolic acids), organic acids, essential oils, vitamins, and other fat-soluble compounds. Vascular plants are rich in physiologically active compounds, which affect the development of plant communities. Extract-based bioproducts do not pollute the environment, and their use as phytochemicals is a promising direction for organic farming. According to a number of researchers (Russo and Berlyn 1991, Ortuño et al. 2016, Van Oosten et al. 2017), biostimulants offer an alternative to synthetic chemicals, which are quickly becoming obsolete, and can contribute to the development of sustainable agriculture. It is noteworthy that in recent years there has been an increased interest in studying the possibilities of using plant extracts in agricultural practice in various countries. For example, foliar feeding of the pea with *Moringa oleifera* leaf extract increased the yield and dry weight of seeds, the content of protein and photosynthetic pigments, especially after treatment with a 4% solution of the extract (Merwad 2018), as well as the number of pods, the dry matter content, the weight of seeds, and the growth rate (Abohassan and Abusuwar 2018). M. A. Sayed et al. (2016) demonstrated that a 5% aqueous extract from *Leonurus siribicus* had a growth-promoting effect on the seed germination and seedling growth of wheat. Biologically active extracts from seaweeds have been used in agriculture as biostimulants to improve water and nutrient uptake and increase stress tolerance and yield (Russo and Berlyn 1991, Poincelot 1993, Nabti et al. 2017, Saudi 2017). Different concentrations of extracts from *Reynoutria japonica* Houtt. (1–5%) were reported to improve the germination rate and seed vigor of wheat, barley, and soybean (Borovaya et al. 2020).

Phenolic compounds, including flavonoids, are of great importance for plant growth and development. They play an active role in the physiological processes occurring in plant cells and can influence plant reproduction, promote root formation, inhibit the development of pathogens, and regulate oxidative phosphorylation. They are universal multifunctional adaptogens to adverse environmental factors (Zaprometov 1993, 1996, Prusakova et al. 2008, Lakhanov et al. 2001, Mishina et al. 2010, Makarenko and Levitsky 2013, Nefedieva et al. 2013, Mierziak et al. 2014). Synthetic analogs have been created based on these natural plant growth regulators and widely used in agricultural production to increase the productivity of plants and improve the quality of grown products (Prusakova and Chizhova 2005, Brunoni and Vegyari 2007, Belopukhov et al. 2013).

Buckwheat (*Fagopyrum esculentum* Moench) is a plant species with an exceptional ability to produce polyphenolic secondary metabolites such as flavonoids, including rutin, which has antioxidant, angioprotective, antibacterial, and hepatoprotective properties (Kreft et al. 1999, 2002, 2006, Fabjan et al. 2003, Wieslander et al. 2005). According to some studies, flowers of *F. esculentum* contain 8.3–10% flavonoids, and leaves – 1.2–2.6% (Vysochina 2004). It was discovered that leaves and flowers together contain an average of 63.2–76.5% of the total rutin in *F. esculentum* plants, and stems – 7–15.7% (Polekhina 2013). A. G. Klykov et al. (2018) determined that a larger amount of rutin was contained in buckwheat flowers (47–63 mg/g), a much smaller amount – in stems (6–14 mg/g), and the smallest amount – in roots (3–8 mg/g). Flavonoid compounds are known to accumulate in buckwheat vegetative parts while plants grow and develop and reach the highest level during the period from flowering to fruiting, and then gradually decrease as fruits set (Gorkova 2002, Metzger 2010, Pavlovskaya et al. 2010, Anisimova 2011, Klykov et al. 2018). This may happen due to changes in the physiological and biochemical state of plants at the beginning of the generative period of the ontogeny (Polekhina 2013). Thus, buckwheat can be considered a promising source of biologically active compounds. The use of *F. esculentum* for medicinal purposes and preventive care requires the rutin content to be as high as possible. At the same time, the flavonoids isolated from buckwheat can be an alternative to synthetic biostimulants in agriculture, but this issue has not been studied in depth.

Our research aim was to study the antioxidant activity and plant growth-promoting effect of the extracts obtained from the aboveground parts of *F. esculentum* plants with different stem colors, as well as to evaluate the influence of the extracts on the accumulation of flavonoid compounds in buckwheat fruits in Primorsky kray, Russia.

Materials and methods

Obtaining biologically active compounds (BACs)

Buckwheat varieties Izumrud, Pri 7 (Fig. 1), Bashkirskaya krasnostebel'naya (red-stemmed), and Primorochka were used in the experiment and grown under field conditions in Primorsky kray. The aboveground parts of *F. esculentum* plants with different stem colors (green, green-red, red-green, and red) were collected at the flowering stage, dried in a darkened well-ventilated room, ground down, mixed

with a 70% aqueous solution of ethanol, and boiled for one hour. The solvent was removed at reduced pressure and a dry residue of BACs was obtained.

Figure 1. Stem color (SC) of *F. esculentum* plants (a – green, b – green-red, c – red-green, d – red)

Determining the content of phenolic compounds

Phenolic compounds were extracted from seed samples and the aboveground parts of buckwheat plants with 70% ethanol. The extraction was carried out in a flask attached to a reflux condenser and heated in a boiling water bath for one hour. After the extraction, the extract was cooled and filtered through a paper filter. The filtered liquid was used for the spectrophotometric study of phenolic compounds. The total flavonoid content was determined with a 2% solution of AlCl_3 at a wavelength of 413 nm (Vysochina 2004). The spectrophotometric studies were carried out using a spectrophotometer Shimadzu UV mini-1240 (Japan). Calibration curves were plotted on the basis of rutin ("Chemapol", Czech Republic), expressing the content of these compounds as %.

Measuring the antioxidant activity

The antioxidant activity of the extracts was measured spectrophotometrically using a stable free radical 1,1-Diphenyl-2-picrylhydrazide (DPPH; $\text{C}_{18}\text{H}_{12}\text{N}_5\text{O}_6$, $M = 394.33$) (Blois 1958). A DPPG solution was prepared in methyl alcohol at a concentration of 750 μM . The experimental extracts were obtained by dissolving the dry residue of BACs in methanol at concentrations of 100, 10, and 1 $\mu\text{g}/\text{ml}$. In our study, 120 μl of the extracts dissolved in methanol and 30 μl of 750 μM DPPG in methanol were pipetted into a 96-well plate and left for one hour in the dark with stirring at room temperature. The antioxidant activity was measured on a Multiskan FC flatbed spectrophotometer (Termo Scientific, Canada) at $\lambda = 517 \text{ nm}$. All the experiments were carried out with three repetitions; the calculations were performed using the SigmaPlot software.

Assessing the growth regulatory activity of BACs

Laboratory experiment. The technique of seed germination in a filter paper roll was employed to assess the biological effect of the extracts on root formation in buckwheat seedlings (Chaikina et al. 2009). The aqueous extracts at concentrations of 1, 10, and 100 $\mu\text{g}/\text{ml}$ were prepared beforehand. Dry seeds of variety Izumrud were laid out on strips of filter paper 12x42 cm in size, moistened with a certain extract according to the experimental variant, rolled up, placed in vessels with a small amount of the tested solution (100 ml), and left in a thermostat at 26-27°C for three days. After the incubation, the length of the main root of the seedlings was measured. The control seedlings were grown in distilled water. The research results were calculated as the arithmetic mean of the three repetitions (25 seeds each). Reagent-grade rutin was used as the standard.

Field experiments. Released buckwheat variety Izumrud served as the research object. The area of one experimental field plot was 15.0 m^2 ; the positioning of the plots was random. The experiment was carried out with four repetitions. The effect of the extracts from *F. esculentum* plants with different stem

colors on the content of flavonoids in buckwheat fruits was evaluated at extract concentrations of 1, 10, and 100 µg/ml. Buckwheat plants were treated at a rate of 30 ml/m² at the beginning of bud formation. In the control variant, plants were not treated with any extract. Harvesting operations started when 75–80% of the plants reached the maturation stage. The analysis of the flavonoid content in the buckwheat fruits was performed after the harvested crops were brought to meet the quality standard.

Statistical analysis

The STATISTICA 6 (Halafian 2007) package was employed for the statistical processing of the research data. Analysis of variance was used to determine significant differences between the mean values of the treatments at a significance level of 0.05.

Results

Study on the flavonoid content in the buckwheat extracts

In search of the natural sources of BACs, we evaluated the content of flavonoids in the aboveground parts of buckwheat varieties Izumrud and Pri 7 collected at the stages of flowering and maturation. The research results showed that plants of both varieties with red stems and branches contained more flavonoids (3%) than plants with green ones ($P < 0.05$) (Fig. 2). Unlike variety Izumrud, variety Pri 7 showed significant differences in the accumulation rate of flavonoid compounds in plants with red, red-green, and green-red stems – 3.0, 2.6, and 2.3%, respectively. Varietal differences in the rutin biosynthesis were revealed. Plants of variety Izumrud, excluding plants with red stems, accumulated more flavonoid compounds (by 1.1–1.2 times) than variety Pri 7 ($P < 0.05$).

Antioxidant activity of the buckwheat extracts

Our study established that the extracts from the aboveground parts of the buckwheat plants collected at the flowering stage had pronounced antioxidant activity. Additionally, the antioxidant activity increased with a rise in the extract concentration reaching its highest values at the 100% concentration and being almost at the level with the reference solution of rutin (Fig. 3, 4).

There were only insignificant differences in the antioxidant activity of the extracts from the raw materials with different stem colors – from red to green ($P < 0.05$) – at the highest concentration. An exception was the variant with plants of variety Izumrud with green stems, where the antioxidant activity was significantly lower.

At all other experimental concentrations (1–10%), the extracts from the vegetative parts of buckwheat plants with red stems had a slightly higher antioxidant activity, which gradually decreased in the following order: red-green stems – green-red stems – green stems. This could be explained by differences in the flavonoid content in these plant groups as shown in Fig. 2. A significant difference in the antioxidant activity of the extracts was noted between the variants with red-stemmed plants and the

group consisting of plants with green-red and green stems ($P < 0.05$). No significant varietal differences in the antioxidant activity were found in any of the experimental variants.

Effect of the extracts on the root formation in *F. esculentum* plants

According to our research data, the strongest growth-promoting effect on the root formation in variety Izumrud was produced by the 0.1-1.0% extracts from buckwheat variety Bashkirsкая krasnostebel'naya. The root length of the seedlings was 65.0–67.0 mm in these variants, which was very close to the effect produced by the reference solution of rutin (66.0-70.2 mm at concentrations of 0.001-10.0%), significantly exceeded the control ($P < 0.05$) (Table 1).

Table 1
Effect of the extracts from *F. esculentum* varieties with different stem color on the root formation in buckwheat seedlings (Mean $\pm$ SE)

Extract variant	Root length, mm					
	Extract concentration (%):					
	Control (without processing)	0.001	0.01	0.1	1.0	10.0
Rutin (reference)	59.0 $\pm$ 0.3	66.0 $\pm$ 0.4*	66.0 $\pm$ 0.5*	70.2 $\pm$ 0.6*	69.6 $\pm$ 0.5*	65.2 $\pm$ 0.6*
<i>variety Izumrud</i>						
Red SC	59.3 $\pm$ 0.2	61.6 $\pm$ 0.4*	63.4 $\pm$ 0.4*	62.2 $\pm$ 0.2*	62.3 $\pm$ 0.4*	62.2 $\pm$ 0.4*
Red-green SC	59.5 $\pm$ 0.3	61.8 $\pm$ 0.3*	63.7 $\pm$ 0.4*	63.0 $\pm$ 0.4*	63.1 $\pm$ 0.4*	62.3 $\pm$ 0.4*
Green-red SC	59.3 $\pm$ 0.3	61.1 $\pm$ 0.5	59.3 $\pm$ 0.3	59.4 $\pm$ 0.3	58.1 $\pm$ 0.3	60.5 $\pm$ 0.3
Green SC	59.5 $\pm$ 0.2	59.8 $\pm$ 0.4	61.5 $\pm$ 0.3*	61.6 $\pm$ 0.5*	61.6 $\pm$ 0.4*	60.9 $\pm$ 0.3
<i>variety Pri 7</i>						
Red SC	59.5 $\pm$ 0.3	62.4 $\pm$ 0.3*	63.0 $\pm$ 0.4*	63.1 $\pm$ 0.5*	62.4 $\pm$ 0.5*	62.3 $\pm$ 0.5*
Red-green SC	59.3 $\pm$ 0.3	61.6 $\pm$ 0.5*	62.6 $\pm$ 0.5*	61.1 $\pm$ 0.4	60.4 $\pm$ 0.2	58.1 $\pm$ 0.3
Green-red SC	59.3 $\pm$ 0.3	58.7 $\pm$ 0.3	59.3 $\pm$ 0.4	61.1 $\pm$ 0.5	59.8 $\pm$ 0.3	59.9 $\pm$ 0.4
Green SC	59.4 $\pm$ 0.3	61.2 $\pm$ 0.4	61.7 $\pm$ 0.3	61.2 $\pm$ 0.5	60.6 $\pm$ 0.3	58.8 $\pm$ 0.3
<i>variety Primorochka</i>	59.2 $\pm$ 0.2	59.2 $\pm$ 0.3	57.4 $\pm$ 0.2	59.8 $\pm$ 0.3	60.4 $\pm$ 0.3	58.0 $\pm$ 0.2
<i>variety Bashkirskaya krasnostebel'naya</i>	59.0 $\pm$ 0.3	61.0 $\pm$ 0.2	63.0 $\pm$ 0.4*	65.0 $\pm$ 0.4*	67.0 $\pm$ 0.5*	63.0 $\pm$ 0.5*

* Indicates a significant difference between the control group and the group treated with the extract ($P < 0.05$)

Compared to the control, the root length increased by 9.2–10.2%, respectively. Slightly lower values were detected in the case of the 0.01-10.0% extracts from plants of variety Izumrud with red and red-green stems (62.2–63.7 mm) and from plants of variety Pri 7 with red stems (62.4–63.1 mm). These results

were significantly higher than the control as well (by 4.9–7.1%). The extracts from plants with green and green-red stems had a weaker growth-promoting effect (at the level of the control group). It should be emphasized that the obtained results correlated with the flavonoid content (Fig. 2).

Effect of the extracts from *F. esculentum* on the flavonoid content in buckwheat fruits

To study the effect of the extracts from the aboveground parts of *F. esculentum* plants with different stem colors on the accumulation of flavonoids in buckwheat fruits, plants were treated with these extracts at the stage of bud formation. The results are presented in Table 2.

Table 2

Effect of the extracts from *F. esculentum* plants with different stem colors on the flavonoid content in buckwheat fruits (Mean $\pm$ SE). *P* values were < 0.05

Extract concentration	The content of flavonoids in buckwheat fruits, %	
Control (without treatment)	0.11 $\pm$ 0.01	
Rutin (reference)	0.12 $\pm$ 0.01	
	Extract from the vegetative parts of variety Izumrud	Extract from the vegetative parts of variety Pri 7
	Red SC	
1 μ g/ml	0.20 $\pm$ 0.02	0.14 $\pm$ 0.01
10 μ g/ml	0.21 $\pm$ 0.02	0.16 $\pm$ 0.02
100 μ g/ml	0.22 $\pm$ 0.02	0.22 $\pm$ 0.02
	Red-green SC	
1 μ g/ml	0.17 $\pm$ 0.02	0.11 $\pm$ 0.01
10 μ g/ml	0.18 $\pm$ 0.02	0.12 $\pm$ 0.01
100 μ g/ml	0.20 $\pm$ 0.02	0.15 $\pm$ 0.01
	Green-red SC	
1 μ g/ml	0.11 $\pm$ 0.01	0.12 $\pm$ 0.01
10 μ g/ml	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01
100 μ g/ml	0.15 $\pm$ 0.01	0.14 $\pm$ 0.01
	Green SC	
1 μ g/ml	0.11 $\pm$ 0.01	0.11 $\pm$ 0.01
10 μ g/ml	0.12 $\pm$ 0.01	0.11 $\pm$ 0.01
100 μ g/ml	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01
<i>F</i>	0.032	0.031

The highest flavonoid content in the fruits of buckwheat variety Izumrud (0.20–0.22%, which significantly exceeded the control) was noted in the variant with the extract obtained from *F. esculentum* plants with red stems at all the studied concentrations (1-100 μ g/ml) ($P < 0.05$).

The less intense the anthocyanin coloration of buckwheat stems was, the less effective the extracts obtained from them were. Thus, the extracts prepared from plants with red-green stems had a slightly weaker effect. The plants treated with these extracts accumulated 0.17–0.20% flavonoids, while the

other extracts (from plants with green-red and green stems) did not affect the flavonoid content. A similar correlation was observed in the case of variety Pri 7. A significant difference from the control was noted in the variants with the extract (at concentrations of 1-100 µg/ml) prepared from plants with red stems (0.14–0.22% flavonoids) and with the extracts (at a concentration of 100 µg/ml) from plants with red-green and green-red stems (0.14–0.15%). It should be highlighted that the accumulation rate of flavonoid compounds in buckwheat fruits were affected by the type of the raw materials used for preparing the extracts. For example, the vegetative parts of variety Izumrud were more effective. According to the results of the conducted research, a patent "Method for increasing the flavonoid content in buckwheat fruits" was obtained (Patent No. 2729743, the Russian Federation 2020).

Discussion

Research on the flavonoid content and antioxidant activity of the buckwheat extracts

Flavonoids are responsible for the color of plants (Vysochina 2004). Therefore, it is not surprising that buckwheat plants with anthocyanin (red) coloration of stems and branches (e.g. the studied *F. esculentum* varieties Izumrud and Pri 7) are characterized by a higher content of flavonoid compounds compared to plants with green stems. Differences in the content of rutin, which is the main flavonoid in buckwheat, depend on the color of plants as has been described by Klykov et al. (2018). They have found out that the rutin content in the aboveground parts of buckwheat plants directly correlates with the intensity of the anthocyanin coloration of stems. Based on these data, a new method was developed for breeding *F. esculentum* varieties with an increased content of rutin (Patent No. 2255466, the Russian Federation, 2005). A. Kosyan et al. (2010) have discovered the same correlation. The revealed varietal differences in the rate of flavonoid synthesis between varieties Izumrud and Pri 7 were confirmed by our previous studies (Klykov et al. 2018). The rutin content was 38.1 mg/g in variety Izumrud exceeding variety Pri 7 by 18%.

Plants of *F. esculentum* varieties Pri 7 and Izumrud with red stems and, thus, with a higher content of flavonoids, had the highest antioxidant activity, which directly correlated with the intensity of anthocyanin coloration. This is due to the fact that phenolic compounds, including flavonoids, are able to inactivate free radicals and act as substrates for peroxidases protecting plants against reactive oxygen species (Prusakova et al. 2005, Fuchsman et al. 2005, Mishina et al. 2010, Nefedieva et al. 2013). The highest antioxidant activity is characteristic of 100% buckwheat extracts, which are almost as effective as the reference solution of rutin. The antioxidant properties of flavonoids have been described by many researchers (Terao et al. 1994, Andersen and Markham 2005, Muzafarov et al. 2010, Brunetti et al. 2011). V.T. Metzger et al. (2010) have shown that the antioxidant activity against hydroperoxyl radicals increases along with an increase in the content of phenolic compounds, including rutin, during the growing season of buckwheat and is the highest in seven weeks after sowing. The experiments of

Abbasi et al. (2015) have proved the high antioxidant activity of 100% extracts from *F. esculentum* seeds (81.64%).

Effect of the buckwheat extracts on root formation and flavonoid biosynthesis in *F. esculentum* plants

Incorporating biologically active compounds in the technology of buckwheat cultivation is an effective way to increase the productivity and improve the quality of the crop as well as to enhance its resistance to abiotic and biotic environmental factors. Buckwheat extracts with a high content of flavonoid compounds might be a promising sustainable means of promoting plant growth and development. The extract obtained from the aboveground parts of buckwheat plants had a noticeable effect on root formation in *F. esculentum*. The strongest growth-promoting effect on the buckwheat root system was produced by the 0.1-1.0% extracts from variety Bashkirskaya krasnostebel'naya and varieties Izumrud and Pri 7 with red and red-green stems. Thus, the more flavonoids plants contained, the higher the growth-promoting effect of the extract obtained from them was. Some studies have confirmed that phenolic compounds have a beneficial effect on the growth and development of plants and their organs (Kefeli 1974, Phenolic... 2015). It should be noted that the concentrations of the extracts below 0.1% were not effective enough and an increase in the concentration up to 10% did not produce a stronger positive effect on root formation in plants.

Treating buckwheat plants with the extracts from *F. esculentum* at the stage of bud formation increased the accumulation rate of flavonoids in buckwheat fruits. The highest flavonoid content was detected in the fruits of variety Izumrud (0.20–0.22%) in the variant with the extract from the raw materials with red stems at all the studied concentrations. This might be associated with an increased content of biologically active compounds, particularly flavonoids, in plants of this variety. The effect of plant extracts containing BACs and extract-based bioproducts on the qualitative composition of agricultural produce has been reported by many researchers. For example, the content of flavonoids was on average by 65% higher in the roots of the maize plants grown from the seeds that had been treated with an aqueous extract from *Salvia officinalis* in comparison to the control. The flavonoid content in the roots of the seedlings was by 30–78% higher (Elshafey et al. 2014). It has been demonstrated that extracts from fennel and lemon residues and brewer's spent grain produce a growth-promoting effect on *Solanum lycopersicum* increasing not only the growth rate of its shoots and the total yield of tomatoes but also the content of mineral oil, vitamin C, and phenolic compounds (Abou Chehade et al. 2018). The phenolic compounds (phenol carboxylic acids, rutin, quercitrin, and quercetin) isolated from the aboveground parts of buckwheat plants increase the content of flavonols in *F. esculentum* (Kiselev et al. 1985). The amaranth plants grown from the seeds treated with bioproducts (betacyanin and polyphenols) accumulate more flavonoids in their leaves (by 12–32%) than the control specimens (Gins et al. 2010).

Conclusion

F. esculentum plants, especially those with red and red-green stems, are active producers of essential biologically active compounds, particularly flavonoids. Extracts from buckwheat can be used in

agriculture as plant growth regulators and facilitate the growth of plant roots and the accumulation of flavonoid compounds in buckwheat fruits. Buckwheat extracts might be recommended as effective antioxidants. Using *F. esculentum* extracts obtained from plants with red and red-green stems as biostimulants is a promising direction for organic agriculture. Buckwheat can become an excellent raw material for the production of biostimulants on an industrial scale due to its wide distribution, high plasticity, and a rapid growth rate as well as its natural ability to acquire anthocyanin coloration after exposure to adverse environmental factors, such as heightened levels of solar radiation.

Declarations

Conflict of interest. The authors declare no conflict of interest.

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Figures

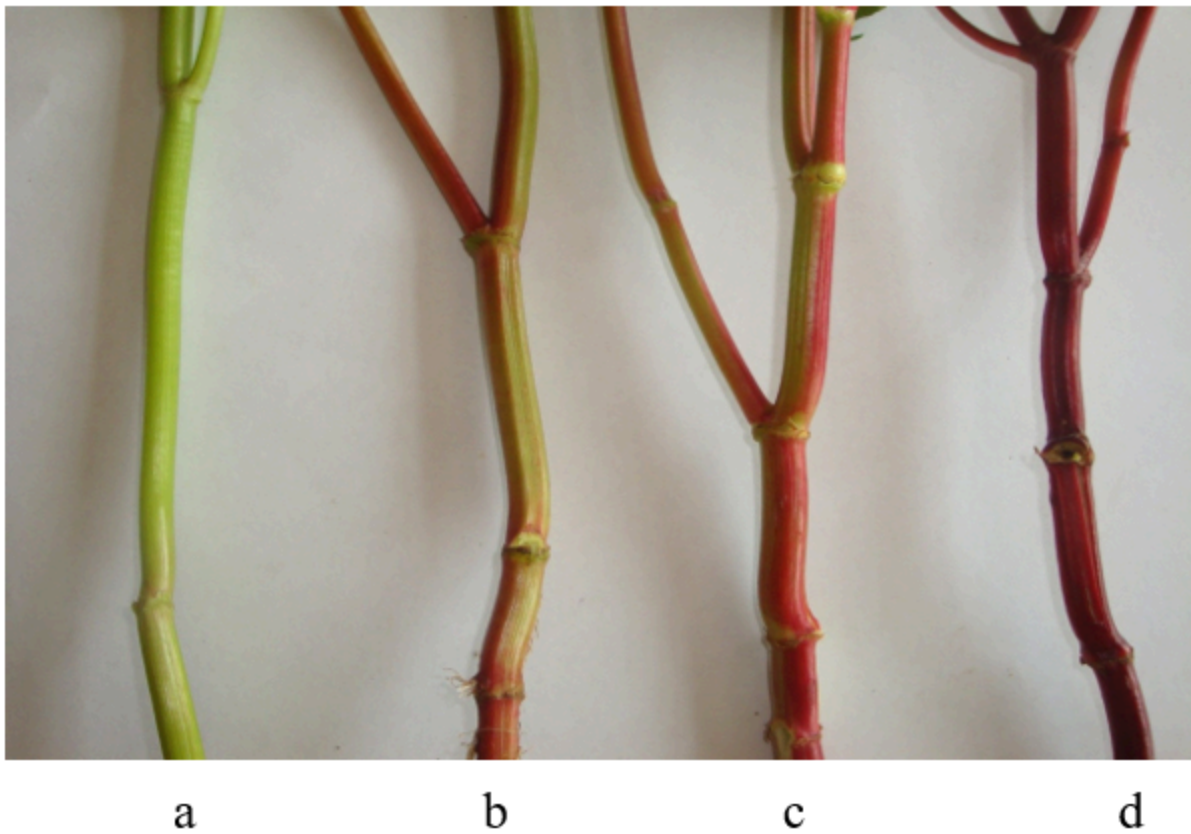


Figure 1

Stem color (SC) of *F. esculentum* plants (a – green, b – green-red, c – red-green, d – red)

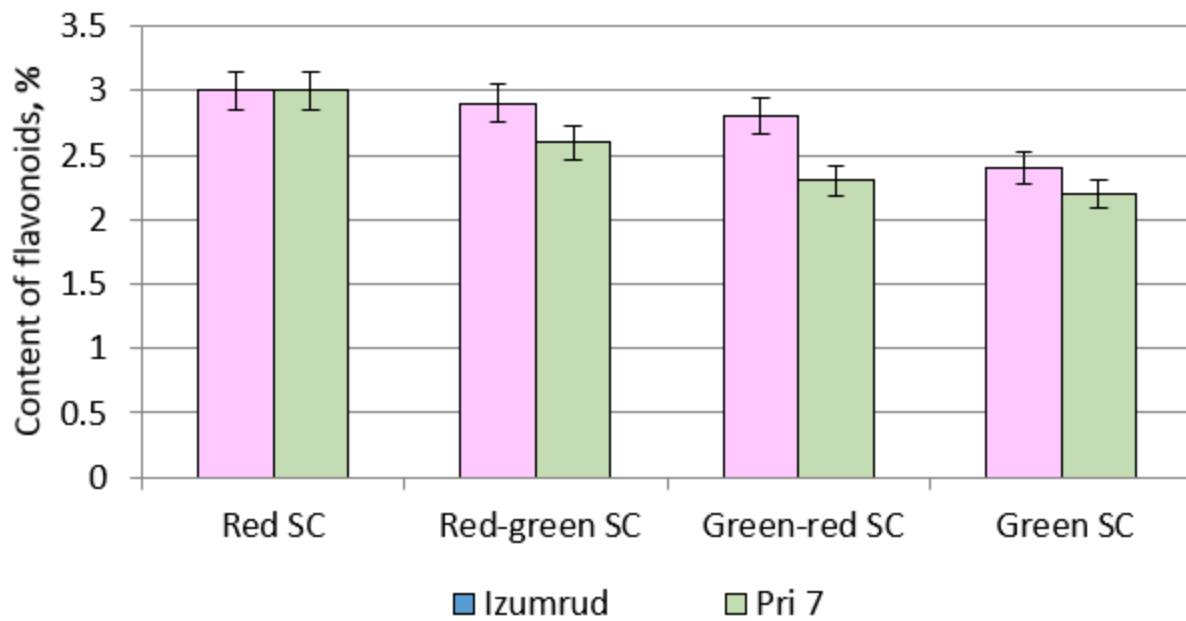


Figure 2

The content of flavonoids in the extract from the buckwheat aboveground parts depending on the variety and stem color (SC)

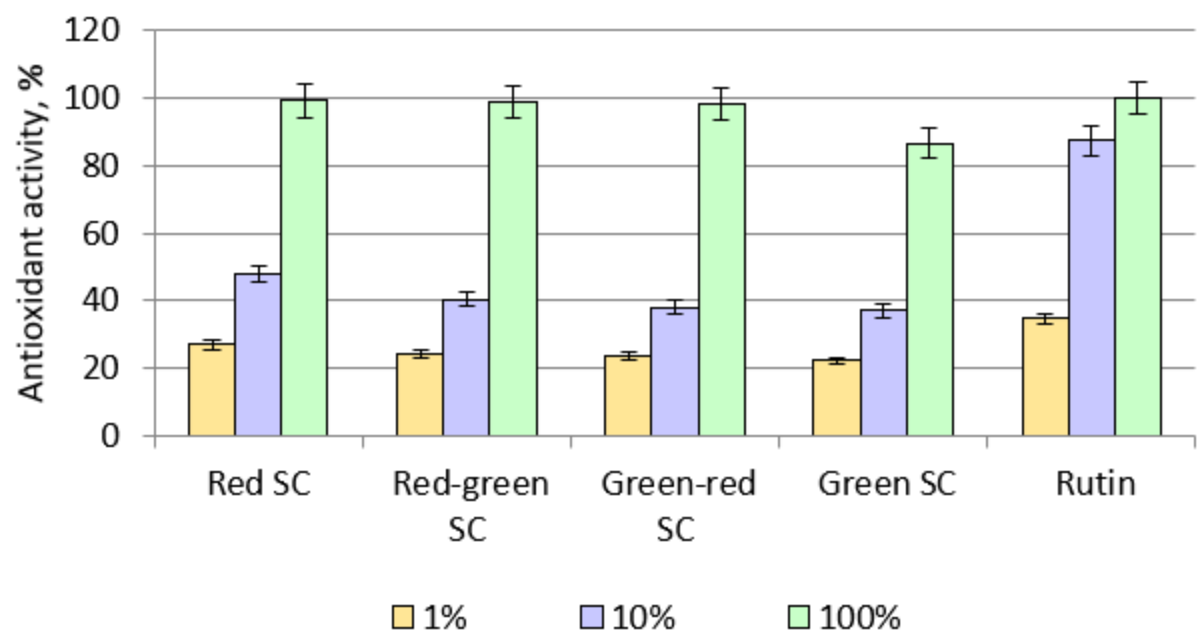


Figure 3

Antioxidant activity of the extracts from buckwheat vegetative parts (variety Izumrud) depending on the stem color

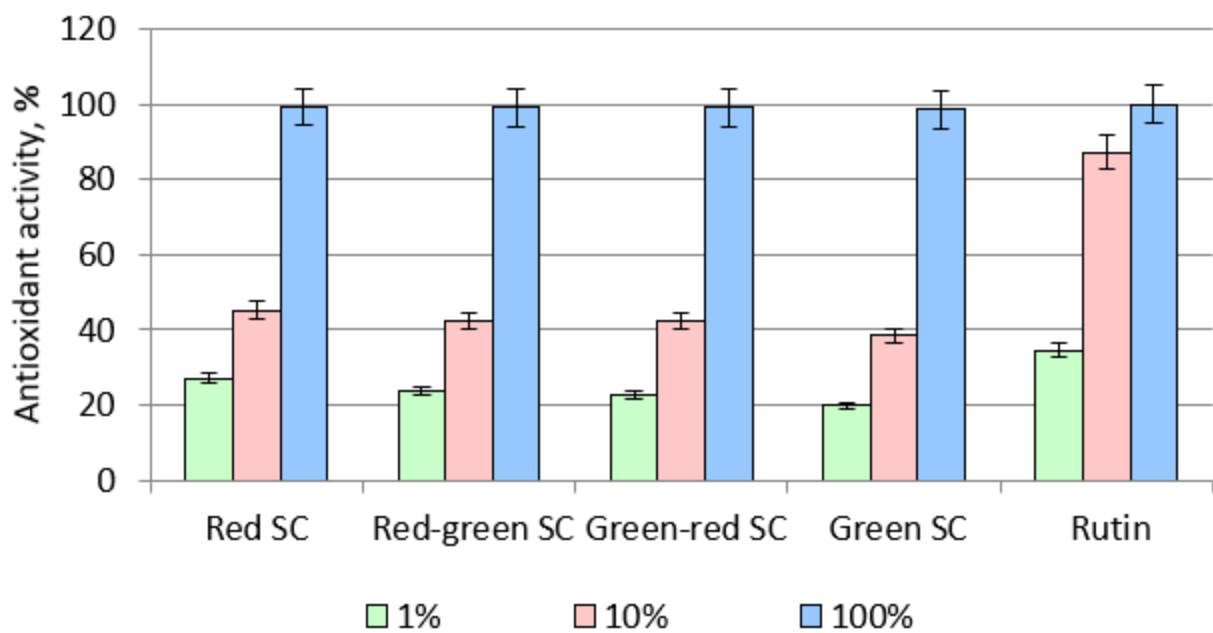


Figure 4

Antioxidant activity of the extracts from buckwheat vegetative parts (variety Pri 7) depending on the stem color