Accumulation of phenolic antioxidants in flowers and fruits of guelder rose (Viburnum opulus L.) depending on site conditions

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Abstract. Various parts of guelder rose (Viburnum opulus L.), including the bark, leaves, flowers, and fruits, have long been used in modern and folk medicine. This study aims to evaluate how site climatic conditions and soil properties influence the accumulation of phenolic antioxidants in flowers and fruits of Viburnum opulus L. It was established that the content of total phenolics in fruits was 1.7 times that in the flowers; the content of tannins, 2.5 times; antioxidant activity, 2.3 times. A high correlation between antioxidant capacity and the content of phenolic compounds (r = 0.90; p < 0.001) and tannins (r = 0.94; p < 0.001) was revealed. Site conditions have a strong effect both on the accumulation of secondary metabolites in Viburnum opulus L. and on the antioxidant activity of the study sample extracts. The highest concentrations of the phytocomponents under study were associated with heavy precipitation and high average contents of organic matter and macroelements (phosphorus, potassium, and nitrogen). These findings highlight the importance of fulfilling agro-technical requirements for the cultivation of Viburnum opulus L., both to ensure high yields and to obtain products with better biochemical composition.

1. Introduction
Guelder rose (Viburnum opulus L.) is a fast-growing shrub up to 4.5 m tall, common in Europe, North and Central Asia, North Africa, and North America. Despite their astringent taste, fruits of guelder rose are a traditional ingredient in Russian, Ukrainian, and Siberian cuisine, used in making jelly sweets, jams, liqueurs, and herbal teas [1].

Different parts of guelder rose, including the bark, leaves, flowers, and fruits have been long used in modern and folk medicine. Particularly, the fruits are used to treat cardiovascular, lung, kidney, and stomach diseases [2]. The medicinal properties of different parts of the plant are accounted for by phenolic compounds. The fruits contain coumaryl-quinic and chlorogenic acids as well as proanthocyanidin B2. Flavonoid derivatives (kaempferol-3-glucoside and kaempferol-3,7-glucoside) are dominant compounds in the flowers [3].

Phenolic compounds, which are secondary metabolites of plants, are responsible for both medicinal and organoleptic properties of guelder rose products, primarily, their colour and taste. Although there is a wide variety of phenolic compounds, most studies focus on flavonoids, stilbenes, lignans. It has been established that many individual compounds comprising these classes have antioxidant activity, i.e. they can scavenge and reduce free radicals and other active forms of oxygen [4].

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It is widely recognised that the synthesis and accumulation of secondary metabolites, including phenolic compounds and other antioxidants, in plants is a dynamic process that undergoes changes during ontogenesis and is affected by various factors of the site environment (geographical, climatic, edaphic, orographic, and biotic ones) [5]. A combination of ontogenetic and environmental factors ultimately determines the chemical composition of medicinal plants as well as its variability.

This study aims to explore how phenolic antioxidant accumulation in flowers and fruits of guelder rose depends on the site climatic and edaphic conditions.

2. Materials and methods
The study focused on flowers and fruits of guelder rose collected from three experimental plots (EP1–EP3) in the Kaliningrad region from May through August 2018. Five shrubs were selected in each plot to harvest composite samples for bioactive substance analysis.

Site conditions were determined based on climatic and edaphic indicators. Soil samples were taken at a depth of 30 cm. Tests to measure pH_KCl, content of organic matter, of phosphorus (P_2O_5), of potassium (K_2O) and of nitrogen were performed in line with the state standards [6-9].

To analyse phenolic compounds and antioxidant activity, the plants were dried at 60°C and cut into ≤1mm sections. Bioactive components were extracted using 70% aqueous ethanol (1:20 w/w). The extracts were filtered, and the filtrate was used for analysis.

The total content of phenolic compounds was determined using the Folin–Ciocalteu method; of tannins, using the ferrocyanide method [10]. These contents were expressed as mg gallic acid equivalent per 1g dry weight.

The ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was measured to evaluate antioxidant activity. The extract-DPPH solution mixture was incubated in the dark at room temperature for 30 min. A decrease in optical absorption, as compared with control (96% aqueous ethanol), was registered at 517 nm. An ascorbic acid solution was used as a standard [11].

The data were statistically processed using OriginPro 2019b software (OriginLab Corporation, US). To ascertain if there were significant differences between the experiment types, two-way analysis of variance (ANOVA) was performed. Tukey’s HSD test was employed as a criterion of significance at p<0.05. Correlation analysis used the Pearson coefficient. The charts below show average values and the standard deviation (n=5). Letters indicate significant differences between experimental plots for both the flowers and the fruits (p<0.05). Asterisks mark significant differences between the flowers and the fruits (p<0.001).

3. Results and discussion
Our analysis showed that the fruits of guelder rose had a higher total phenolic and tannin content than the fruits did, regardless of the collection site (figures 1–2).

The lowest total content of both phenolic compounds and tannins was observed in the fruit and flower samples harvested in EP2. The content of phenolic compounds differed significantly across the three plots, whereas the content of tannins in flowers from EP1 and EP3 showed no statistical difference. This may be indicative that the biosynthesis of this class of compounds is not as labile as thought previously, and that changes in the total content of phenolics are associated with the effect of site conditions on other phenolic derivatives (flavonoids, phenolic acids).

Antioxidant activity is a composite indicator influenced by the presence of various chemical compounds, including phenolic derivatives, ascorbic acid, reduced glutathione, and antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, etc.) in the extract. This study measured the antioxidant activity of the extracts of flowers and fruits of guelder rose based on their ability to scavenge DPPH radicals. It was established that antioxidant capacity was higher in the extracts of guelder rose fruits than in those of the flowers (figure 3).
Correlation analysis showed a strong correlation between antioxidant capacity and the content of phenolic compounds ($r=0.90; p<0.001$) as well as of tannins ($r=0.94; p<0.001$). The findings suggest that those components strongly affect the antioxidant properties of the fruits and flowers of guelder rose.

It was demonstrated that the site environment had a major effect on the accumulation of secondary phenolic metabolites in flowers and fruits of guelder rose. A recognised decisive factor in the accumulation of bioactive substances by plants is the balance between heat supply and humidity. In extreme climatic conditions, antioxidant components are used up to combat free radicals and other active forms of oxygen as well as to mitigate the consequences of oxidative stress, particularly, lipid peroxidation. Within favourable temperature and humidity ranges, the active synthesis of phenolic compounds, which continues throughout the reproductive growth stage, more than makes up for their consumption. Thus, the content of phenolics remains consistently high in the plants. Table 1 shows the climatic characteristics of the study areas. A distinctive characteristic of EP3 was heavy precipitation during both flowering and fruiting. A recent study of spinach (*Spinacia oleracea* L.) plants has shown that extreme precipitation enhances phenolic concentrations in leaves [12]. On the other hand in some other studies the total content of phenolic compounds and content of tannins increased in plants grown in dry conditions with water deficiency [13, 14].

Site-dependence of antioxidant capacity is also the case for guelder rose. The highest antioxidant capacity was associated with the extracts of the fruits harvested from EP3. This finding is in good agreement with the results of correlation analysis.
Table 1. Climatic characteristics of the study areas at different phenological stages.

| Phenological stage | Study area | Average temperature, °C | Precipitation, mm | Average wind speed, m/s |
|--------------------|------------|--------------------------|-------------------|-------------------------|
|                    | EP1        | 20                       | 57                | 3.0                     |
| Flowering          | EP2        | 18                       | 45                | 1.2                     |
|                    | EP3        | 21                       | 78                | 3.1                     |
|                    | EP1        | 15                       | 72                | 2.1                     |
| Fruiting           | EP2        | 15                       | 67                | 4.3                     |
|                    | EP3        | 16                       | 97                | 3.5                     |

Table 2 shows the results of soil acidity and macroelement analysis for the study areas. It was established that, in soil from EP2, the content of organic matter was 3.8–4.2 times lower than in the other plots; of phosphorus, 1.4–2.3 times; of potassium, 5.1–6.9 times; of nitrogen, 4.6–5.0 times. EP1 had a higher phosphorous and potassium content that EP3 did.

Table 2. A comparison of soil composition in the experimental plots.

| Study area | pH_{KCl} | Organic content, % | Phosphorus (P_{2}O_{5}) content, mg/kg | Potassium (K_{2}O) content, mg/kg | Total nitrogen content, % |
|------------|----------|--------------------|----------------------------------------|-----------------------------------|---------------------------|
| EP1        | 7.0      | 3.14               | 1202                                   | 700                               | 0.23                      |
| EP2        | 7.1      | 0.83               | 524                                    | 101                               | 0.05                      |
| EP3        | 6.9      | 3.51               | 758                                    | 512                               | 0.25                      |

The analysis of correlation between the content of bioactive components in the fruits and flowers of guelder rose, on the one hand, and the chemical properties of EP soils, on the other, showed a direct correlation between them (r=0.63-0.96; p<0.05).

Mineral nutrition, particularly microelement composition, is a major factor affecting phenolic content in plants through enzymes involved in biosynthesis. Moreover, phenolics contribute to cell protection from oxidative stress, which occurs, for instance, when certain elements of mineral nutrition are deficient in soil [15].

A lower phytocomponent content observed in samples from plots with smaller concentrations of organic matter, phosphorous, potassium, and total nitrogen is explained by both the reduced activity of enzymes (phenylalanine ammonia-lyase, chalcone synthase) in the biosynthesis of phenolic compounds and the consumption of phenolic compounds in cell protection from oxidative stress.

4. Conclusion

The study demonstrated that the fruits of Viburnum opulus L. were a more valuable source of phenolic compounds as well as tannins and had a higher antioxidant capacity as compared to the flowers. Analysis of variance showed that site conditions had a significant influence on the accumulation of bioactive components in both flowers and fruits of guelder rose. A lack of macromolecules in soil was associated with lower contents of phenolic antioxidants in flowers and fruits of the guelder rose. The findings highlight that, to ensure high yields and to obtain products with better biochemical composition, it is critically important to fulfil agro-technical requirements for the cultivation of Viburnum opulus L.

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