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The Content of Phenolic Compounds in *Gentiana cruciata L.* Growing in the Territory of the Republic of the Tatarstan

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ABSTRACT

The article presents the results of a study of the quantitative content of phenolic compounds in aerial shoots and underground organs of the cruciate gentian (Gentiana cruciata L.) growing in plant communities on the territory of Tatarstan. The quantitative analysis of medicinal plant materials carried out by the spectrophotometric method confirmed the results of earlier studies, according to which the aerial part of the cruciform gentian plants is 5-6 times richer in biologically active phenolic compounds than rhizomes with roots. In addition, it was found that plants growing on the territory of Laishevsky (meadow cenosis) and Chistopol districts (forest cenosis) of the Republic of Tatarstan accumulate different amounts of biologically active compounds, which is probably due to the ecological-cenotic growing conditions. Namely, the positive effect of the concentration of some elements of mineral nutrition (exchangeable potassium, mobile phosphorus) and the insolation level, as well as the negative influence of organic matter (humus) on the content of the sum of xanthones and flavones in the plant raw materials of the cruciform gentian was noted. It was found that the maximum content of phenolic compounds among all studied samples of raw materials is typical for the aboveground part of Gentiana cruciata plants collected on the territory of Laishevsky district.

Key words: *Gentiana cruciata L*, *Cruciate gentian*, Phenolic compounds, Xanthones, Flavones

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INTRODUCTION

Cruciform gentian-Gentiana cruciata L., gentian family (*Gentianaceae*) is a perennial herb with a thick rhizome [1]. It is known as one of the analogues of the medicinal plant, gentian yellow (*Gentiana lutea L.*), the root part of which (*Gentianae radices*) is widely used in the world both in official and in alternative medicine [2].

Gentians are known to be rich in biologically active substances (BAS), especially bitter glycosides, therefore, traditionally decoctions, tinctures and water extracts of these plants were used as agents that improve digestion and enhance food absorption in diseases of the gastrointestinal tract (loss of appetite, dyspepsia, achilia) [3,4]. Several other therapeutic effects, such as antitumor, antiviral, immunomodulating, hepatoprotective, and antioxidant effects, are mainly due to phenolic compounds that accumulate more actively in aerial plant shoots: Flavones from the flavonoid group and xanthones [5-7].

Since yellow gentian does not grow on the territory of Russia [2], it is of interest to study the domestic species, cruciform gentian, which quantitative composition of medicinal plant materials is exactly unknown.

The objective of the research is to study the accumulation of phenolic compounds in plant samples of the cruciform gentian plant communities of the Republic of Tatarstan, depending on the ecological-cenotic growing conditions.

METHODS

The object of the study was the grass and rhizomes with roots of Gentiana cruciata collected on the territory of the Republic of Tatarstan in 2019 and manually harvested. The collection of raw materials was carried out in the forest phytocenosis of the Chistopol and clover-

meadow cenosis of the Laishevsky districts in compliance with the general rules of collection [8]. The aboveground part of the middle-aged generative plant (g2) of the cruciform gentian was collected during the flowering period, the underground part of the postgenerative plant was collected in September after the aboveground part died off.

The raw material was dried by air-shadow drying with active air ventilation at 40-60oC.

The quantitative content of xanthone and flavone compounds was determined by spectrophotometry [9,10].

Quantitative determination of the amount of xanthones. About 0.8 g (accurately weighed) of the crushed raw material is placed in a 100 ml flask with a thin section, and 50 ml of 60% alcohol is added. The flask is weighed with an error of ±0.01 g, connected to a reflux condenser and heated in a water bath for 2 hours. Then the flask is cooled to room temperature and weighed, brought to the initial weight with 60% alcohol. The contents of the flask are filtered through a folded paper filter discarding the first 25 ml of filtrate (solution A).

2.0 ml of the resulting solution A was placed into a 25 ml volumetric flask, 3 ml of aluminum chloride in 2% alcohol solution is added, and the volume of the solution is diluted to the mark with 96% alcohol and mixed (solution B).

After 30-40 minutes, the optical density of solution B is measured on a spectrophotometer at 410 nm in a quartz cuvette with a layer thickness of 10 mm. As a reference solution, a solution consisting of 2.0 ml of solution A and 0.1 ml of concentrated acetic acid, diluted to the mark with 96% alcohol to the mark in a 25 ml volumetric flask, is used.

The content of the sum of xanthones in terms of alpizarin in dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{A \cdot 25 \cdot 50 \cdot 100}{E^{1\%}_{1\,cm} \cdot a \cdot 2 \cdot (100 - W)}$$

Where, A - the optical density of solution B; "E" _"1 cm" ^"1%" - specific absorption index of the complex of alpisarin with aluminum chloride at 410 nm, equal to 239; a - weight of raw materials, g; W - moisture content of raw materials, %.

Quantitative determination of the amount of xanthones. About 0.2 g (accurately weighed) of the crushed raw material is placed in a 100ml flask with a thin section, and 40 ml of 60% alcohol is added. The flask is connected to a reflux condenser and heated in a water bath at 60 oC for 30 minutes. Then the flask is cooled to room temperature and filtered through a cotton swab into a 100 ml flask. 40 ml of 60% alcohol is added to the meal, the flask is connected to a reflux condenser and again heated in a water bath at 60°C for 10 minutes. Then the flask is cooled to room temperature. The contents of the flask are filtered through a folded paper filter. Bring the volume of the solution to the mark with 60% alcohol, rinsing the flask and filter.

5.0 ml of the filtrate is placed in a 25 ml volumetric flask and evaporated to dryness under reduced pressure. 10 ml of a mixture of 10 volumes of methanol and 100 volumes of glacial acetic acid (solution A) was added to the residual volume. Then another 10 ml of a solution was added, consisting of 25 g/l boric acid in anhydrous formic acid and 20 g/l oxalic acid in anhydrous formic acid. The volume of the solution is dilluted to the mark with anhydrous acetic acid and mixed (solution B).

After 30 minutes, the optical density of solution B is measured on a spectrophotometer at 401 nm in a quartz cuvette with a layer thickness of 10 mm. As a reference solution, a solution A was used, diluted with 10 ml of anhydrous formic acid, and diluted to the mark in a 25 ml volumetric flask with anhydrous acetic acid.

The content of the sum of flavones in terms of vitexin in dry raw materials in percent (X) is calculated by the formula:

$$X = \frac{A \cdot 0.8}{a}$$

Where, A - the optical density of the test solution; a - sample of raw materials, g.

The statistical significance of the differences between the differentiating groups was determined using the Student's t-test. Differences were considered statistically significant at $p < 0.05$. The graphs show the arithmetic mean values of the definitions and their standard errors.

RESULTS AND DISCUSSION

As follows from the data obtained (Figure 1),

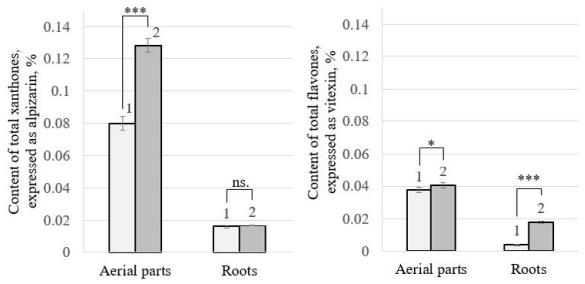

Figure 1: The content of xanthones and flavones in the aerial parts and roots of plants of Chistopol (1) and Laishevsky (2) districts. The differences are statistically significant at p <0.05 (*); p <0.001 (***).

grass samples collected in Laishevsky district contain significantly more both the amount of xanthones in terms of alpizarin (p <0.001) and the number of flavones in terms of vitexin (p <0.05) than samples of the Chistopol region. This statement is also true for the level of flavone compounds in underground organs (p <0.001); at the same time, the rhizomes of plants of both collection sites are characterized by an almost equal content of xanthones (p >0.05), which indicates that the accumulation of biologically active substances in the underground part is independent of external conditions. It should be noted that the content of xanthone compounds in underground organs (0.0167) is 3 times lower than the data described in the literature (0.05%) [11].

The highest content of both the sum of flavones (0.0406%) and the sum of xanthones (0.1283%) is noted in the extracts of herbal medicinal plant samples from Laishevsky district, and the latter value is 46.5% lower than the minimum known from the literature [12]. Based on the foregoing, it can be assumed that there is a somewhat limited possibility of using cruciform gentian as a plant substitute for yellow gentian as a source of phenolic substances.

The performed correlation analysis showed that the more flavones or xanthones are found in the aboveground part, the correspondingly higher is their content in underground organs (r=0.52). It should also be noted that in the underground part the total amount of phenolic compounds is 5-6 times lower than in the aboveground one. This is consistent with previous studies [6,7].

The relationship between the total content of phenolic substances and the area of plant growth is shown: the samples of the Laishevsky district (0.207%) are 1.5 times richer in biologically active substances than the samples of the Chistopol district (0.134%).

Comparison of the data on the accumulation of flavones and xanthones with the degree of soil fertility (Table 1) revealed that soil samples from Laishevsky region contain 3 times more exchangeable potassium and 1.7 times more mobile phosphorus than samples from Chistopol region, and, probably, therefore, plants accumulate more phenolic compounds (r = 1). This fact underlines the marked influence of these indices of the edaphic factor on the content of phenolic compounds noted in other studies and confirms the results of earlier studies [13]. A negative correlation (r=–1) is observed when comparing the level of accumulation of biologically active substances with the content of humus: more phenolic compounds accumulate in samples of plants growing on soils poor in humus.

Table 1: Results of agrochemical analysis of soil samples.

Agrochemical indicators/ Place	Laishevsky district, meadow	Chistopol district, broad-leaved forest
pHsalt, unit	5,8	5,5
Humus, %	2,7	4,7
Nitrate nitrogen, mg/kg	16,6	24,6
Ammonia nitrogen, mg/kg	14,5	11,25
Mobile phosphorus, mg/kg	135	80
Exchangeable potassium, mg/kg	190	66
Exchangeable calcium, mmol/100 g	15,0	22,25
Exchangeable magnesium, mmol/100 g	3,75	5,0

Thus, the maximum content of biologically active substances was identified in the aboveground shoots of samples from Laishevsky district collected in open areas; therefore, plants in illuminated habitats (clover meadow cenosis) accumulate more phenolic compounds than plants growing in shaded conditions (broad-leaved forest). This confirms the assumption that light stimulates the biosynthesis and accumulation of several secondary metabolites [14].

SUMMARY

The study showed that the aboveground shoots of Gentiana cruciata harvested in phytocenoses of two regions of Tatarstan accumulate 5-6 times more phenolic compounds than in underground organs.

The accumulation of phenolic compounds positively correlates with the content of mobile phosphorus and exchangeable potassium in the soil, and negatively - with humus.

The level of accumulation of biologically active substances is higher in plants of the meadow cenosis with high insolation, and lower in forest plants.

The maximum number of phenolic substances is contained in the aerial shoots of the cruciform gentian collected in the Laishevsky region of the Republic of Tatarstan.

CONCLUSIONS

Thus, the quantitative content of phenolic substances, xanthones and flavones, in the plant raw material of Gentiana cruciata, depending on the ecological and cenotic growing conditions, has been established.

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