Flavonoid content and antioxidant activity of extracts of Polygonum Weyrichii Fr. Schmidt

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Abstract. This work presents the results of the use of maceration and ultrasonic extraction for the isolation of antioxidant substances, particularly flavonoids, from the Polygonum Weyrichii Fr. Schmidt growing in the Murmansk region. This plant is characterized by a high content of flavonoids and other antioxidant substances, and therefore may be of interest as a potential source of natural antioxidants. The efficiency of extracting flavonoids from various parts of plants (inflorescences and leaves) by maceration for 24 hours in 70% ethanol and using ultrasonic extraction for 1-5 hours at a temperature of 50 °C is compared. Antioxidant activity was evaluated for all extracts using the phosphomolybdate method. It was found that the maximum extraction of flavonoids is achieved by using ultrasonic extraction for 2 to 4 hours.

1. Introduction

One of the promising sources of herbal remedies is medicinal plants containing flavonoids. Flavonoids are a large group of polyphenols exhibiting both anti- and prooxidant activity, widely distributed in plants and representing an important component of the mechanism of non-specific adaptations to adverse environmental conditions [1]. The use of flavonoids in medical practice is justified by the uniformity of membrane mechanisms of damage and adaptation in plants and animals [2]. It is known that in plants, under the influence of extreme factors, the content of flavonoids and other low molecular weight compounds can increase significantly [3]. The territory of the Murmansk region is beyond the Arctic Circle, the specificity of its conditions is a combination of adverse factors of natural and anthropogenic origin. Taking into account these factors, it is logical to assume that the Arctic regions can serve as one of the most important sources of pharmaceutically valuable medicinal plants. Among the plants growing in the Murmansk region, the most promising source of flavonoids is the plant Polygonum weyrichii Fr. Schmidt, which was introduced to the Kola Peninsula in the 1920s.

The genus Polygonum, first described by Linnaem, is the largest genus of the buckwheat family (Polygonaceae) and includes 200 species, widespread throughout the globe. Many species of this genus are medicinal plants and are used in medicine. These are Polygonum aviculare, Polygonum persica, Persicaria hydropiper and many others. P. Weirich was first found in the middle of about Sakhalin was a participant in the expedition of Admiral Putyatin, a doctor by Weirich in 1854, in whose honor the name was given. The boundaries of the range over the past 35-40 years have expanded significantly. It has been successfully introduced in the Leningrad, Moscow, Murmansk, Novgorod regions, in the Republic of Belarus, the Baltic states, Western Siberia, and in some areas it is used as a fodder plant [4, 5].
Polygonum weyrichii is grassy, polymorphic, polycarpic, early vegetation, fast-growing, winter-hardy perennial plant (figure 1).

![Polygonum weyrichii plants](image)

**Figure 1.** Polygonum weyrichii plants.

It is known P. weyrichii contains the flavonoid: avicularin, hesperidin, quercetin, hyperoside, quercetin-3-rhamnosid, kaempferol, myricetin, rutin, epigallocatechin, epicatechin [6–8]. These flavonoids have the following types of pharmacological activity: antioxidant [9, 10], angioprotective [11], diuretic [12], antithrombogenic [13], anti-inflammatory [14], antiviral [15], anticarcinogenic [10] and many others [16, 17].

Representatives of this species have effectively naturalized in local conditions and are now widely distributed throughout the industrial part of the region. Since these plants are perennial, this allows the use of plantations without reseeding for 15-20 years. P. weyrichii characterized by high productivity of green mass, in the Murmansk region these plants reach a height of 3 meters, their tissues are rich in flavonoids [18]. In our study, we used samples of plants collected in the territories of Polar Alpine Botanical Garden and Institute (PABGI KSC RAS) in Apatity and Kirovsk. The Kirovsk Territory, located about 200 m above the Apatity, is distinguished by a more severe and less continental climate, shorter vegetation season and a more pronounced wind load, thus, the climatic conditions in Kirovsk are more extreme.

The aim of the present work was to study the efficiency of extracting flavonoids from various organs of P. weyrichii plants collected in different climatic conditions using two extraction methods: maceration for 24 hours in 70% ethanol and using the method of exposure to ultrasound for 1–5 hours at a temperature of 50 °C and determination of the antioxidant activity of the obtained extracts.

2. Materials and methods

2.1. Materials
Plant samples were collected in Apatity and Kirovsk in July 2018. They are inflorescences of plants grown in Apatity (Sample 1), inflorescences of plants grown in Kirovsk (Sample 2), and leaves middle tiers of plants grown in Apatity (Sample 3). To ensure representative sampling, 2 kg of different plant organs were collected. The P. weyrichii was identified by experienced biologists from the PABGI KSC RAS. Drying and storage were carried out in accordance with the rules for drying and storage of vegetable medicinal raw materials [19]. Then the plant material was powdered, passed through a sieve with a diameter of 1.0 mm and dried for 3 hours at a temperature of 60 °C to stabilize its mass. All
samples were used with a LOD (loss on drying) of not more than 10%. 70% ethanol with water (v/v) of medical grade (RFK Company) was used as a solvent.

2.2. Methods
Two extraction methods were used: maceration at room temperature for 24 h, and ultrasound-assistant extraction (UAE) at 50 ºC for 1 – 5 hs. Plant material was mixed with solvent in the ration 1:10 w/v and preliminarily shaken before extraction procedure. Extracts were filtrated and centrifuged using ELMI Multi Centrifuge CM 6M at 2000 rpm for 15 min.

Flavonoids content was estimated using complexation reaction of flavonoids with aluminum chloride [20]. 0.05 ml of the extract was added to the measuring tube, 0.1 ml of a 2% solution of AlCl3 in 96% ethanol was added, and the volume was adjusted to 2.5 ml with ethanol of the same concentration. In the control sample, 1-2 drops of 30% acetic acid were added to 0.05 ml of the extract, and then the volume was brought up to 2.5 ml. The solutions were mixed and after 40 min the absorbance at 415 nm of the solution with aluminum chloride was measured using a KFK-3-01 “ZOMZ” spectrophotometer in a cuvette with a layer thickness of 0.5 cm, using a solution with acid for comparison. The total content of flavonoids (in% by weight of air-dried raw materials) was determined by the formula

\[ w_{\text{flavonoids}} = \frac{y \times V_1 \times V_2}{M \times V_3 \times 10^6} \times 100\% \]

were \( y \) – flavonoids content in 1 ml of tested solution, followed by calibration in the equivalent concentration of rutin, µg;

- \( V_1 \) – extract volume, ml;
- \( V_2 \) – dilution volume, ml;
- \( V_3 \) – analyzed sample volume, ml;
- \( M \) – mass of dried plant material, g.

Evaluation of total antioxidant capacity of extracts was estimated using phosphomolybdate method [21] spectrophotometrically. Reagent solution of 4 mM of ammonium molybdate, 28 mM potassium phosphate and 0.6 M sulfuric acid was used. Solutions of ascorbic acid in 70% ethanol in concentrations 100 – 1000 µg/ml were prepared for calibration. For each concentration 0.2 ml of calibration solution was mixed with 2 ml of reagent solution in a sealed vial and incubated at 95 ºC for 90 min. A blank solution contained 2 ml of reagent solution and the appropriate volume of the same solvent. Then absorbance at 850 nm was measured.

The same technique was used to evaluate total antioxidant capacity of extracts. But if 0.2 ml of the crude extracts are directly mixed with reagent solution, absorbance will be out of calibration range, therefore dilution was needed. In order to determine the necessary degree of dilution, samples of extracts diluted 10, 20, 30, and 40 times were prepared. It was found the 30 times dilution is the most convenient for measurements. Total antioxidant capacity was evaluated in the equivalent of ascorbic acid concentration.

The yield of antioxidant substances in terms of equivalent ascorbic acid content in the dried plant material (mg/g) can be calculated by the formula

\[ w_{\text{antioxidants}} = \frac{V_{\text{extract}} \times C_{\text{antioxidants}}}{m_{\text{plant}}} \text{ mg/g} \]

\[ C_{\text{antioxidants}} = 30 \times k \times A_{850}, \text{ mg/ml} \]

were \( k \) – calibration coefficient, \( A_{850} \) – absorbance at 850 nm, 30 – dilution, \( V_{\text{extract}} \) – volume of crude extract, \( m_{\text{plant}} \) – mass of dried plant material.
3. Results and discussion
All obtained data are presented in a table 1.

Table 1. Flavonoids containing and total antioxidant capacity of extracts of P. weyrichii.

| Sample | Extraction type | Time (h) | W antioxidants (mg/g) | W flavonoids (mg/g) |
|--------|----------------|----------|-----------------------|---------------------|
| 1      | UAE            | 1        | 12.86                 | 4.3                 |
|        |                | 2        | 12.43                 | 3.7                 |
|        |                | 3        | 11.57                 | 4.3                 |
|        |                | 4        | 12.37                 | 4.2                 |
|        |                | 5        | 14.39                 | 4.1                 |
|        | Maceration     | 24       | 23.94                 | 4.6                 |
|        |                | 1        | 27.31                 | 6.3                 |
|        |                | 2        | 30.80                 | 6.9                 |
| 2      | UAE            | 3        | 29.02                 | 7.1                 |
|        |                | 4        | 29.20                 | 6.2                 |
|        |                | 5        | 27.37                 | 5.8                 |
|        | Maceration     | 24       | 24.06                 | 5.9                 |
|        |                | 1        | 12.55                 | 3.4                 |
|        |                | 2        | 12.73                 | 3.6                 |
| 3      | UAE            | 3        | 13.16                 | 3.8                 |
|        |                | 4        | 18.31                 | 3.5                 |
|        |                | 5        | 12.55                 | 3.3                 |
|        | Maceration     | 24       | 23.08                 | 4.2                 |

The results for total flavonoid content are presented in a figure 2.

Figure 2. Flavonoids content in the extracts.
The results for of total antioxidant capacity of extracts are presented in a Figure 3.

![Figure 3. Total antioxidant capacity in the equivalent content ascorbic acid.](image)

It can be seen, the inflorescences of P. Weyrichii collected in Kirovsk exhibit a higher antioxidant capacity in comparison with other samples. Also UAE for 2 – 4 h is better for antioxidants extraction. But for inflorescences and leaves of P. Weyrichii collected in Apatity maceration allows to achieve a higher yield of antioxidant substances. This may be due to the fact that different sets of substances, not just flavonoids, are responsible for the antioxidant activity of extracts of different samples.

4. Conclusions
Two methods of extraction antioxidant substances from P. Weyrichii, total flavonoid containing and total antioxidant capacity evaluation are presented in this work. Maceration and ultrasound-assistant extraction were applied to obtain extracts. It was obtained that extracts of inflorescences of P. Weyrichii exhibit higher antioxidant capacity and flavonoid containing then leaves. The optimal extraction conditions are followed: temperature is 50°C, time of extraction is 2 – 4 h, ratio of the plant material mass to the extractant volume 1:10. But also maceration for 24 at room temperature may be applied for antioxidant isolation from the leaves. Despite this, the UAE seems more time-effective then maceration.

Obtained data can be used for the development of technology of antioxidant additions, nutrients and cosmetics production using P. Weyrichii as antioxidant substances source.

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