Herbaceous plants growing in Arctic zones as potential perspective sources of valuable flavonoids

A Korovkina, V Zhirov, N Tsvetov and D Petrashova

Federal Research Centre “Kola Science Centre of the Russian Academy of Sciences”, Apatity, Russian Federation

E-mail: dinapetrashova@mail.ru

Abstract. Flavonoids represent a wide group of polyphenols involved in many key processes of plant growth and its can be successfully used in medical practice. In plants that are exposed to extreme factors of various nature, the flavonoids synthesis occurs more intensively compared with plants of the same species growing under more favorable conditions. Thus, it can be assumed that it is the Arctic plants that can be important sources of flavonoids. This paper presents the results of a search for flavonoid sources among herbaceous plants growing in the Arctic zone, in particular, the Murmansk region. The total flavonoid content in ethanol extracts of 24 plant species was estimated using the reaction of complexation with aluminum chloride. It was shown that Lathyrus pratensis L., Lupinus arcticus L., Hedysarum alpinum L., Chamaenerion angustifolium L., and Polygonum weyrichii species contain the highest amounts of flavonoids.

1. Introduction
Flavonoids are a wide group of polyphenols involved in many key processes of plant growth. The most important role is played by flavonoids in the mechanism of non-specific adaptation of plants to adverse environmental factors, such as high-intensity visible light, ultraviolet radiation, heat stress, increased concentrations of heavy metals, pathogenic microorganisms, etc. [1, 2]. Since flavonoids exhibit high antioxidant activity [3], they protect plants from free radical oxidation processes that underlie damage to all cellular life forms [4].

Plant flavonoids are successfully used in medical practice [5, 6]. In addition to antioxidant activity, flavonoids have the potential to inhibit the onset and development of inflammatory diseases [7], can modulate a number of key elements in the cellular signal transduction pathways associated with apoptosis, angiogenesis, and metastasis, they inhibit the growth of cancer cells and angiogenesis and induces apoptosis of cancer cells, thus exerting an anticancerogenic effect [8]. Since flavonoids are widespread, primarily in land plants [9], it is land plants that are the main natural source of these compounds.

The works of various authors found that in plants that are exposed to extreme factors of various nature, the synthesis of flavonoids occurs more intensively compared to plants of the same species from regions with more favorable conditions [10–12]. In particular, extreme conditions are inherent in the Arctic zones. The climate of the Arctic is characterized by a lack of heat, high humidity and extreme lighting conditions associated with the polar day and polar night [13]. In this regard, we hypothesized that plants growing under extreme conditions of arctic latitudes are potential sources of flavonoids for the needs of pharmaceuticals and the production of biologically active additives. Creation of appropriate
technologies requires data on the seasonal dynamics of flavonoid accumulation by various plant species, on which plant organs accumulate more target substances, on the environmental and age dependences of this process and the development of methods for the efficient extraction of flavonoids.

In this work, the following goals are set: Firstly, a preliminary determination of the flavonoid content in various aerial organs of herbaceous plants growing in the Murmansk region, as one of the industrially developed Arctic regions, in which adverse conditions of high latitudes are combined with intense anthropogenic impact.

2. Materials and methods

2.1. Materials
Aluminium chloride, acetic acid (all with > 99% purity, Vekton, Russia), ethanol of medical grade (96%, RFK Company, Russia), and distilled water were used in present work. Leaves and inflorescences of plants of Amaryllidaceae, Apiaceae, Asteraceae, Caryophyllaceae, Fabaceae, Geraniaceae, Onagraceae, Plantaginaceae, Polemoniaceae, Polygonaceae, Polypodiopsida, Rosaceae, Ranunculaceae, Urticaceae, and Valerianoidae families growing in the Polar Alpine Botanical Garden-Institute were collected to estimate total flavonoid content. All samples were dried and stored in accordance with the rules for the drying and storage of vegetable medicinal raw materials [14]. The dried plant material was powdered, passed through a sieve with a diameter of 1.0 mm and additionally dried for 3 hours at 60 °C to stabilize its mass.

2.2. Extraction procedure
Reflux extraction with 70% (v/v) ethanol-water mixture was used to estimate total flavonoid content (TFC). The mass:volume ratio of plant material and the solvent was 1:100.

2.3. Total flavonoid content
A method based on the complexation reaction of flavonoids with aluminum chloride was applied to determine total flavonoid content [15, 16]. 0.05 ml of extract was mixed with 0.1 ml of 2% solution of AlCl₃ in 96% ethanol, and volume was adjusted to 2.5 ml with 70% ethanol. Absorbance at 415 nm of the analyzed solutions was measured using a KFK-3-01 “ZOMZ” spectrophotometer. The calibration curve was prepared using solutions of rutin in 70% ethanol-water mixture (100 – 1000 µg/ml). TFC is calculated by the formula

\[ w_{\text{flavonoids}} = \frac{k \times A_{415} \times V_1 \times V_2}{M \times V_3 \times 10^6}, \text{ mg/g} \]

where \( k \) – calibration coefficient, \( A_{415} \) – absorbance at 415 nm, µg; \( V_1 \) – extract volume, ml; \( V_2 \) – dilution volume, ml; \( V_3 \) – analyzed sample volume, ml; \( M \) – mass of dried plant material, g.

All analyses were performed in three replications. The data were analyzed in Statistica 6.0 (StatSoft Inc., USA). The data in the table are presented as x ± SD (mean ± standard deviation).

3. Results and discussion
Estimation of the total number of flavonoids in herbaceous plants of the Murmansk region (table 1) have let to identify the most promising plants: Lathyrus pratensis L., Lupinus arcticus L., Hedysarum alpinum L., Chamaenerion angustifolium L., and Polygonum weyrichii. Also it can be noticed that in the most cases inflorescences contain more flavonoids than leaves.
Table 1. Total flavonoid content (TFC) in vegetative and generative organs of various herbaceous plants (x ± SD, n = 3).

| Plants                      | Parts plants | TFC, % of dry mass |
|-----------------------------|--------------|--------------------|
|                            |              | Apatity districts  |
|                            |              | Kirovsk districts  |
| Amaryllidaceae J.St.-Hil.  |              |                    |
| Allium ursinum L.          | leaves       | 3.84±0.02          |
|                            |              | 3.35±0.03          |
| Allium schoenoprasum L.    | leaves       | 1.27±0.03          |
|                            |              | 2.53±0.04          |
| Apiaceae Lindl.            |              |                    |
| Heracleum sosnowskyi Manden.| leaves       | 4.31±0.04          |
|                            |              | 2.70±0.04          |
| Levisticum officinale W.D.J.Koch | leaves | 4.27±0.02          |
|                            |              | 2.27±0.03          |
| Asteraceae Bercht. & J.Presl nom. cons. | | |
| Arnica montana L.          | leaves       | 1.83±0.04          |
|                            | inflorescences| 2.53±0.04          |
| Solidago virgaurea L.      | leaves       | 1.10±0.03          |
|                            |              | 0.78±0.03          |
| Tussilago L.               | leaves       | 2.31±0.04          |
|                            | inflorescences| 1.01±0.02          |
|                            |              | 0.96±0.03          |
| Caryophyllaceae Juss.      |              |                    |
| Stellaria media (L.) Vill. | leaves       | 1.07±0.04          |
|                            |              | 1.07±0.03          |
| Fabaceae Lindl.            |              |                    |
| Lathyrus pratensis L.      | leaves       | 2.53±0.03          |
|                            | inflorescences| 7.02±0.04          |
| Lupinus arcticus L.        | leaves       | 5.34±0.04          |
|                            | inflorescences| 2.53±0.03          |
|                            |              | 3.73±0.04          |
| Hedysarum alpinum L.       | leaves       | 7.90±0.02          |
|                            | inflorescences| 6.13±0.04          |
|                            |              | 3.81±0.03          |
| Trifolium pratense L.      | leaves       | 2.86±0.03          |
|                            | inflorescences| 3.20±0.03          |
|                            |              | 3.30±0.04          |
| Geraniaceae Juss.          |              |                    |
| Geranium sylvaticum L.     | leaves       | 3.09±0.04          |
|                            |              | 3.16±0.03          |
| Onagraceae Juss.           |              |                    |
| Chamaenerion angustifolium L. | leaves    | 3.98±0.03          |
|                            | inflorescences| 6.10±0.04          |
|                            |              | 4.47±0.04          |
| Plantaginaceae Juss.       |              |                    |
4. Conclusion
The paper presents data on the total content of flavonoids in herbaceous plants growing in the Arctic zone, in particular, in the Murmansk region. Based on the obtained data, it can be concluded that *Lathyrus pratensis* L., *Lupinus arcticus* L., *Hedysarum alpinum* L., *Chamaenerion angustifolium* L., and *Polygonum weyrichii* contain more flavonoids than other species. These plants may be chosen as objects of further thorough researches.

The results are valuable for the development of the use of the biological resources of the Arctic zone of Russia.

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