Content of Coumarins in Various Organs of Sosnovsky’s Hogweed (Heracleum Sosnowski Mandena)

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Abstract. In alcohol extracts of dried shoots, leaves, stems, and seeds of Heracleum sosnowski Mandena collected at different periods of plant growth, the content of coumarins was determined by spectrophotometry. The highest content of coumarins was revealed in seeds. Young shoots contain the least amount of coumarins. The author concludes that for effective use of Heracleum sosnowski Mandena as a source of coumarins it is most beneficial to use its seeds. Consequently, the plant must follow a complete development period. The control of the spread of Heracleum sosnowski Mandena is aimed at destroying the plants before seeds are ripe. Mechanical and chemical methods are intended to destroy plant shoots. Therefore it seems impossible to combine the control of hogweed and the processes of extracting coumarins from it. Special areas are required for hogweed to grow in order to obtain coumarins from it. It is also necessary to provide for strict measures to keep the plant within the territory intended for it. Special requirements are imposed on ecological factors of the territory.

1. Introduction
At present Heracleum sosnowski Mandena is referred to invasive weeds. Its inclusion in the classifier of weeds took place relatively recently, in 2015 [1]. Before that time Sosnovsky’s hogweed was considered as a promising forage crop and it was spreading very quickly over the territories of many regions of the Russian Federation. Breeders worked to increase its size and yield. Varieties were bred that could grow in unfavorable conditions. Now the fight against Sosnovsky’s hogweed is carried out at the state level. The search for the most effective methods of fight against this aggressive plant has accelerated the process of close consideration of its chemical composition.

Coumarins take a special place among the variety of bioactive compounds found in Sosnovsky’s hogweed composition. The world demand for coumarins is growing as new forms of pharmacological drugs, herbicides, insecticides, means of controlling plant diseases are created [2–5]. Special requirements are imposed on coumarins used for treatment of tumor processes in medicine [6, 7], and as anti-tuberculosis drugs [8]. Plants from which coumarines are derived must grow in ecologically favorable areas since the process of purifying coumarins from concomittant bioactive compounds is a very difficult task.

Certain kinds of plants of the Umbelliferae family such as large ammi (Ammi Majus L.) are used to extract coumarins and manufacture pharmacological preparations. Ammifuril (Ammifurinum) drug is a mixture of furanocoumarins: isopimpinellin, xanthotoxin, and bergapten [9]. Ammifuril is derived from seeds of large ammi. Beroxan drug which contains a mixture of bergapten and xanthotoxin is manufactured from fruits of planted parsnip (Pastinaca sativa) of the Apiaceae family. Fruits of drupe
scurfy pea (*Psoralea drupacea*) of the Fabaceae family are used to obtain psoralen drug, a mixture of furanocoumarins: psoralen and isopsoralen.

Due to the growing demand for natural coumarins other plants containing coumarins have been studied. For example, *Ferulago* contains about twenty kinds of pharmacologically active coumarins [10, 11]. Another example is Sosnovsky’s hogweed (*Heracleum sosnowski Mandena*). It is with furanocoumarins contained in it that the danger of the plant sap for human skin is associated. Furanocoumarins are activated by ultraviolet radiation. As a result, there occurs restructuring of the membrane components of skin cells. Consequences of these processes are similar with those of thermal burns [12]. This special feature of hogweed sap aroused the interest of chemists and contributed to a close study of chemical composition of this plant.

Sosnovsky’s hogweed occupies vast areas in Novgorod, Leningrad, Pskov and other districts of the Russian Federation. Various methods to eliminate this plant are applied: mechanical, chemical, agrotechnical. Special technical means, herbicides, human resources are required. Large financial resources are spent on measures to combat Sosnovsky’s hogweed. Due to this there arises a problem of obtaining benefits from this plant to partially compensate the costs. For this purpose bioactive compounds contained in this plant, including coumarins, are studied.

Many researches are engaged with the processes of obtaining coumarins from hogweeds. It has been proven that the content of coumarins in Sosnovsky’s hogweed as in other plants depends on the region of growth, plant species, plant parts taken for analysis, and other factors.

Studies of hogweed’s coumarins are carried out in the chemical laboratory of Yaroslav-the-Wise Novgorod State University. The presence of coumarins in hogweed’s leaves was confirmed by methods of qualitative and quantitative analysis. The dependence of coumarin content on the region of hogweed’s growth was proven by an experimental way. The data on the effect of the extraction method on the final result were obtained [13].

2. Materials and methods

The objects of this research are various organs of Sosnovsky’s hogweed. Plant raw materials for experiments were prepared in the Novgorod area of the Novgorod region on certain days of May-September 2020. Samples of young shoots up to 30 cm high (May 21), leaves, stems and inflorescences (July 16), seeds (September 14) were collected. Samples were collected in compliance with safety requirements, avoiding the ingress of plant sap on exposed skin. Cloudy days were chosen for the work and personal protective equipment was used. The collection of samples was carried out in a territory remote from highways and urban settlements in order to avoid the presence of chemical pollutants in plants that could affect the results of the experiment.

The samples were dried in the shade in the open air and ground in a porcelain mortar to a particle size of no more than 1 mm. The prepared samples were stored without access to air at room temperature.

Coumarins belong to the class of unsaturated lactones of cis-ortho-hydroxyceinnamic acid. They can be extracted with organic solvents. Extraction with ethyl alcohol is the main method for extracting coumarins from plant materials and is widely used in the preparation of medicines [14]. In this work, we used the extraction of coumarins with ethyl alcohol of 95% concentration in a Soxhlet apparatus at a temperature of 80 °C. The extraction time was four hours. The resulting extracts were cooled to room temperature.

The presence of coumarins in alcoholic extracts was confirmed by a lactone test: 5 ml of a 10% KON solution was added to 5 ml of the extract. The mixture was kept in a boiling water bath for 5 minutes. Then a 10% HCl solution was added dropwise. The turbidity of the solution indicated the presence of coumarins in the extract.

For the quantitative determination of coumarins in the obtained alcohol extracts, spectrophotometry was used at a wavelength of 360 nm. To determine the content of coumarins at a given wavelength is recommended by the manufacturers of medicinal alcohol extracts of coumarins and other authors of works on this topic. The measurements were carried out using a UNICO 1201 spectrophotometer. 95% concentration ethyl alcohol was used as a reference solution. We assume that the results may be
insignificantly overestimated due to the presence of organic compounds of other classes similar to coumarins in the alcoholic extract. This fact does not affect the experimental results since a comparative analysis of samples of various parts of the plant was carried out.

3. Results of the research of the content of coumarins
The prepared samples of shoots, leaves and stems, inflorescences and seeds were examined separately. The experiments were carried out with five variants of the material of each plant part. In the obtained alcoholic extracts the presence of coumarins was confirmed using a lactone test and the percentage of coumarins was determined. The experimental results are given in table 1.

Table 1. Coumarin content in Sosnovsky’s hogweed, %.

| sample number | examined plant parts | shoots | leaves and stems | inflorescences | seeds |
|---------------|----------------------|--------|------------------|----------------|-------|
| 1             | 2.8                  | 5.1    | 5.2              | 11.1           |
| 2             | 2.9                  | 4.9    | 5.0              | 11.2           |
| 3             | 3.5                  | 4.9    | 5.0              | 11.0           |
| 4             | 3.4                  | 5.1    | 5.2              | 11.5           |
| 5             | 3.4                  | 4.9    | 5.3              | 11.3           |
| average       | 3.2                  | 5.0    | 5.1              | 11.2           |

As a result of the experiment, a different content of coumarins in the samples of various parts of the Sosnovsky’s hogweed was revealed. Shoots of the plant up to 30 cm high contain fewer coumarins. It should be noted that at this stage of its growth the plant is most often exposed to various means of destroying, including mowing. The coumarin content in mowed mass is small, so to use this raw material to isolate coumarins is not purposeful.

The highest content of coumarins is revealed in seeds. These indicators exceed the minimum values by 3.5 times. It is seeds that are recommended to prepare extracts containing coumarins. It should be noted that seeds of Sosnovsky’s hogweed contribute to its quick dissemination over neighboring territories. They have a good volatility and easily spread by wind over long distances. The seeds remain viable for two years.

Leaves, stems and inflorescences contain approximately the same content of coumarins. During the whole growth Sosnovsky’s hogweed accumulates coumarins in its organs.

The results obtained confirm the general pattern for all plants used to obtain coumarins: it is most effective to take fruits and seeds for this purpose. Sosnovsky's hogweed studied during this experiment is no exception.

4. Conclusion
The research allows us to conclude that Sosnovsky’s hogweed may be used as a source of coumarins. However, to combat hogweed and simultaneously use it as a plant raw materials for extraction of coumarins is not purposeful. If we consider the plant as a raw material for production of medicinal forms of coumarins, then it is more profitable to use seeds. In seeds of Sosnovsky’s hogweed the content of coumarins is about 11%. But to harvest a large number of seeds, it is necessary to specially grow Sosnovsky's hogweed in large areas.

Taking into account that seeds are easily separated from umbrellas and have a high volatility, special equipment will be required to collect raw materials. The boundaries of the plots on which the hogweed grows will need to be constantly monitored, preventing the spread of the plant to neighboring plots.

Leaves, stems and inflorescences of Sosnovsky’s hogweed can be used as a source of coumarins as they contain about 5% of them. The plant under the study is of large size, so leaves and stems are the
main raw material obtained from Sosnovsky’s hogweed.

A plot should be selected very carefully taking into account the environmental situation. The plant absorbs many chemicals from the environment through its large leaves, stems and inflorescences. Later on, when obtaining extracts, chemical compounds can be found in resulting products and affect their biochemical activity.

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