COMPARATIVE PHARMACOGNOSTIC STUDY OF THE ROOTS OF THE MOST COMMON SPECIES OF PLANTS OF THE GENUS ARCTIUM

Tetiana Oproshanska, Olga Khvorost, Kateryna Skrebtsova, Lesia Savchenko, Natalya Fizor

The aim is to carry out a comparative pharmacognostic study of the roots of A. tomentosum and A. minus with the root of A. lappa to confirm or deny their interchangeability.

Materials and methods. A Delta optic BioLight 300 microscope (Poland) was used to study the macro- and microscopic features of plant raw materials. The method of gas chromatography-mass spectrometry was used to identify and quantify organic, including fatty, acids. Quantitative content of amount of organic acids, ascorbic acid, total polyphenols and amount of hydroxycinnamic acids was determined by using spectrophotometry. The content of polysaccharides was determined by the gravimetric method.

The results. For the first time, a comparative morphological and anatomical study of the roots of A. tomentosum and A. minus in comparison with the root of A. lappa was carried out. As a result, a distinctive diagnostic microscopic feature of the roots was established: the shape of the receptacles of the schizogen type. For the first time, the component composition of organic, including fatty acids, for the roots of A. tomentosum and A. minus was determined in comparison with the root of A. lappa, which is the same. The content of 11 organics and 12 fatty acids in plant raw materials of 3 Arctium species was identified and determined. The quantitative content of the amount of organic acids, ascorbic acid, polysaccharides, total polyphenols, and amount of hydroxycinnamic acids in the roots of A. tomentosum and A. minus in comparison with the root of A. Lappa was established, and these indicators are comparable.

Conclusions. For the first time, a comparative pharmacognostical study of the roots of A. tomentosum and A. minus in comparison with the root of A. lappa was carried out which showed minor differences between the roots of these Arctium species and confirms their interchangeability at this stage, especially when harvesting wild plant raw materials, when identification of the species at the botanical level is impossible.

Keywords: root, Arctium, morphological and anatomical structure, identification, organic acids, hydroxycinnamic acids, polyphenols

1. Introduction

According to the website Who plant list, the genus Arctium has 30 species [1]. Plants of the genus are characterized by the similarity of vegetative and generative organs and the ability to form hybrids, which led to a change in the number of species. In recent years, the number of burdock species has increased by 1.5 times [2, 3].

The areas of growth of plants of the genus are Europe, Asia, and North and South America. There are six main species in the Euro-Mediterranean area: A. atlanticum (Pomel) H. Lindb., A. lappa L., A. minus (Hill) Bernh., A. nemorosum Lej., A. palladini (Marcow) R.E Fr. & Soderb. and Arctium tomentosum Mill. [3].

The most common species are A. lappa, A. minus and A. tomentosum [3–5]. Plant raw materials of A. lappa are most often used in medicine. This plant is cultivated in Asia and used in the food industry [3–5]. Plant raw materials of A. minus and A. tomentosum are the smaller used [3].

Plants of the Arctium genus are biennial herbs. They form a basal rosette of leaves in the first year of life and a stem with generative organs in the second [3].

Plants are characterized by a similar morphological structure, which leads to difficulties in species identification. It is especially difficult to identify the species in the first year of life since the main distinguishing features are the structure of the generative organs [2]. One of the options for identification is the study of the genomic type of the species. Such studies for A. lappa, and related species A. tomentosum was conducted by scientists from China. They established distinguishing features between these species at the gene level [6]. Genomic type of A. minus was also studied by other scientists [7], which makes it possible to identify one type of plant from another.

In Ukraine Sokol O. V. studied the biological including morphological features of 4 species of plants of Arctium genus: A. lappa, A. Minus, A. tomentosum and A. nemorosum (resources are insignificant), which grow on the territory of our country. The author paid considerable attention to the study of morphological features of generative organs for the diagnosis of the plant species of Arctium genus. The author also established the morpho-
logical features of the leaf plate of the leaves of the basal rosette of these species of the genus [2].

The main plant raw material which is used in medicine is root. Leaves and seeds are used much less. It was obtained extracts from the root of A. lappa with using different extractants which have anti-inflammatory, antitumor, antioxidant, antibacterial, and antiviral activity [8]. A. lappa has antidiabetic activity by improving glucose homeostasis and reducing insulin resistance [9].

The plant raw material of Arctium genus plants contains lignans. The dominant compound is arctigenin, which shows a therapeutic effect in Alzheimer’s disease, glioma, infectious diseases of the central nervous system, Parkinson’s disease, and epilepsy [10].

The root of A. lappa is a source of prebiotic fibres, chlorogenic acid, cinnarin, lignans and quercetin, and it has antioxidant, anti-inflammatory and hypolipidemic activity [11].

It was isolated and purified polysaccharide from the root of A. lappa, which contains fructose, glucose, galactose and arabinose and its immunomodulatory and inhibitory activity on intestinal inflammation was studied in vitro models [5, 12].

Fischer, S. P. et al. investigated the antinociceptive and anti-edematous effects of extract from A. minus seed in a model of acute gout attack induced by intra-articular injection of monosodium urate crystals. As a result, it was established that seed extract from A. minus at a dose of 100 mg/kg orally reduced mechanical allodynia and prevented mechanical allodynia at doses of 30 and 100 mg/kg. The authors established that allopurinol at a dose of 10 μg/ml and extract from seed A minus at a dose of 10–300 μg/ml inhibited the activity of xanthine oxidase in vitro [13].

Alcoholic and aqueous extracts were obtained from the leaves of A. minus, in which the quantitative content of total polyphenols was determined, and anti-inflammatory, antinociceptive and antioxidant activity was established [14]. From the root, leaves and fruits of A. minus were obtained methanolic, dichloromethane, ethyl acetate, butanol and water extracts in which the quantitative content of total polyphenols, the amount of flavonoids, rutin, chlorogenic, caffeic, coumaric and ferulic acids was determined, and the antioxidant and cytotoxic activity and inhibitory activity of α-aminola, α-glucosidase, lipoygenase and tyrosinase were determined [15].

In the comparative pharmacognostical study of the fruits of A. lappa, A. minus and A. tomentosum were identified 53 compounds which are mainly represented by lignans and fatty acids. Quantification of lignans showed that A. lappa fruits contained less arctigenin but more arctigenin glycoside than A. minus fruits. Linoleic acid was identified in the fruits of A. Minus which was absent in the fruits of A. lappa and A. tomentosum [16].

In 2010, the European Medicines Agency has printed the «Community herbal monograph on Arctium lappa, radix» in which as a plant raw material can be used root of A. lappa, A. minus and A. tomentosum and from related species, hybrids or mixtures thereof [17]. The State Pharmacopoeia of Ukraine (SPhU) 2.1 has been implemented since 2017, and it contains the monograph «Burdock Roots», in which the medicinal plant raw material is the roots of A. lappa, A. minus and A. tomentosum or a mixture of plant raw materials of these types [18]. The monograph «Burdock root» has been implemented in the European Pharmacopoeia (Ph Eur.) 11.3 from January 2024 (before this time it was absent) [19]. According to this monograph, only the root of A. lappa is a plant’s raw material. In SPHU 2.7.2, which will be implemented in July 2024, the monograph «Burdock Root» also offers as a plant raw material only the root of A. lappa [20]. Therefore, the controversy question is which species of plant Arctium genus root should be used as a plant raw material «Burdock Roots»: it is the root of only one of the 3 species of Arctium genus – A. lappa, or it can be a mixture of 3 species plant raw materials. At the same time, the roots of A. minus and A. tomentosum have been studied extremely little. Therefore, a systematic pharmacognostic study of the roots of A. minus and A. tomentosum in comparison to the root of A. lappa is relevant.

The aim is to carry out a comparative pharmacognostic study of the roots of A. tomentosum and A. minus with the root of A. lappa to confirm or deny their interchangeability.

2. Research planning (methodology)

The design of the experiment with a comparative phytochemical characterization of roots of A. minus and A. tomentosum with root of A. lappa included several steps:

- the collection of objects for investigations;
- study macroscopic and microscopic features;
- identification and determine quantitative content of organic acids by GC-MS;
- determine the amount of organic acids, ascorbic acid, polysaccharides, total polyphenols and amount of hydroxycinnamic acids;
- determine differences between roots of three species of plant genus Arctium;
- to show the importance of this research for further study of the plant genus Arctium.

3. Materials and methods

Several samples of root the first year plant of A. minus and A. tomentosum and A. lappa were harvested in autumn in different regions of Ukraine (Table 1).

The study was carried out on the basis of the National University of Pharmacy.

The anatomical structure was studied from freshly harvested fixed and dried plant raw materials [21]. Surface preparations and cross-sections were prepared according to generally accepted methods [22]. The microscopes Delta optical BioLight 300 with camera 2 Mpx (Poland) were used for magnification at 100 and 400 times.

The method of gas chromatography-mass spectrometry (GC/MS) was used for the identification and quantitative determination of organic, including fatty acids. It was obtained methyl ethers from plant raw materials for this research [23]. Quantitative determination of the amount of organic acids, ascorbic acid, total polyphenols and amount of hydroxycinnamic acids was per-
formed by spectrophotometric method on a spectrophotometer Optizen POP (Korea) in accordance with monograph SPhU «Rose fruit» [18], «Roses» [24], «Determination of tannins in herbal drugs» [22] and «Burdock root» [18]. The quantitative content of polysaccharides was determined by gravimetry [25].

4. Results

For the first time, we studied the morphological and anatomical structure of the roots of A. minus and A. tomentosum in the first year of life and carried out a comparative analysis of the root of A. lappa, which was studied by us earlier [26]. Morphological characteristics of the roots of A. minus and A. tomentosum are similar to the roots of A. lappa: the root system is tap, and the roots are straight and cylindrical in shape with rare thin lateral roots. The main root of some plants with a large aerial part may have 1–2 side branches, rarely more. The surface of fresh roots is smooth, dry – wrinkled. Wrinkles were vertical and deep. The colour of the surface is from light brown (fresh root) to dark brown (dried root). The fracture is uneven and granular, from light brown to light yellow-brown in colour. The fracture clearly shows a thin cambium line; the cortex part is wide, and the wood is lighter than the cortex part. At the root of A. lappa, a ring of secretory channels (contents) is present in the cortical part of the fracture [26]. This ring is not characteristic of the roots of A. minus and A. tomentosum.

The anatomical structure was studied using raw materials from freshly harvested plants. But in process of describing results we had same moments which need to rectification and in these cases it was used fixed and dried plant raw materials. At the same time, our research showed that anatomically these types of plant raw materials do not differ.

The anatomical structure of the roots of A. minus and A. tomentosum, like in A. lappa, secondary unbundled. The covering tissue of the periderm is formed by quadrangular parenchyma cells with evenly thickened brown cell membranes, and the cell cavity is colourless (Fig. 1, a, d). Under the covering tissue is a well-developed cortex parenchyma, which is formed by pectoral thick-walled (closer to the periderm) and thin-walled cells. There is tangentially, closer to the covering tissue, a different number of secretory receptacles of the schizogenic type, which are arranged in a circle in the cortical parenchyma of the roots of 3 species of Arctium. The structure receptacles are round in the root of A. minus (Fig. 1, b), and diamond-shaped with elongated corners in the root of A. tomentosum – (Fig. 1, e).

In A. lappa, these receptacles are diamond-shaped (the corners of the diamond are not elongated) and frequent [26]. Sometimes root receptacles A. minus and A. tomentosum contain amorphous brown content of unknown nature, while in the root of A. lappa they are rarely without contents [26]. The phloem is located in narrow radial zones separated by the tips of the pith rays and is formed by thin-walled cells (Fig. 1, a, d). The cambium is thin, one- or two-layered, well defined. The secondary xylem is mainly represented by ladder vessels, wood parenchyma cells and libriform. In the centre of the root are the remains of the primary xylem (Fig. 1, f), in the root A. minus there may also be a small cavity (Fig. 1, c). The cells of the cortical and wood parenchyma of the root of three species of Arctium contain a reserve substance – inulin (Fig. 1, d) [26].

Determination of the component composition of organic, including fatty, acids in the roots of A. minus and A. tomentosum was conducted for the first time. The results of the study in comparison with the roots of A. lappa are shown in Table 2, a sample of GC/MS chromatograms of the compounds of the roots of A. lappa – in Fig. 2.

Table 1

<table>
<thead>
<tr>
<th>Date of harvested</th>
<th>Place of harvested</th>
<th>Coordinates</th>
<th>Name of plant</th>
<th>Kind of research</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.10.2015</td>
<td>Vinnytsia Region</td>
<td>48.494403, 28.444867</td>
<td>A. lappa</td>
<td>Determination of the component composition of organic, including fatty acids</td>
</tr>
<tr>
<td>16.10.2015</td>
<td>Ternopil Region</td>
<td>49.070127, 26.161222</td>
<td>A. tomentosum</td>
<td>Study morphological and anatomical structure</td>
</tr>
<tr>
<td>24.10.2017</td>
<td>Vinnytsia Region</td>
<td>48.225497, 28.696671</td>
<td>A. minus</td>
<td>Quantitative determination of main groups of biologically active compounds</td>
</tr>
<tr>
<td>11.10.2018</td>
<td>Lviv Region</td>
<td>49.977346, 24.068820</td>
<td>A. tomentosum</td>
<td></td>
</tr>
<tr>
<td>17.10.2019</td>
<td>Kharkiv Region</td>
<td>49.890685, 36.608634</td>
<td>A. lappa</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.402099, 36.101028</td>
<td>A. minus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.399678, 36.099429</td>
<td>A. minus</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1 Anatomical features of the root of A. minus (a–c) and A. tomentosum (d–f): a, d – the structure of cortex part; b, e – secretory receptacles of the schizogenic type; c, f – the structure of central part; 1 – inulin
Table 2

The component composition of organic, including fatty acids in the roots of A. minus and A. tomentosum in compared to the roots of A. lappa and quantitative content of components (μg/g, n=5, in terms of absolutely dry plant raw materials)

<table>
<thead>
<tr>
<th>No</th>
<th>The name of the acid</th>
<th>A. minus</th>
<th>A. tomentosum</th>
<th>A. lappa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Oxalic</td>
<td>177.5±0.5</td>
<td>190.2±0.4</td>
<td>173.1±0.6</td>
</tr>
<tr>
<td>2</td>
<td>Malonic</td>
<td>130.4±0.4</td>
<td>111.2±0.2</td>
<td>125.7±0.5</td>
</tr>
<tr>
<td>3</td>
<td>Fumaric</td>
<td>42.5±0.4</td>
<td>48.3±0.3</td>
<td>44.4±0.6</td>
</tr>
<tr>
<td>4</td>
<td>Succinic</td>
<td>1008.3±0.8</td>
<td>864.2±0.6</td>
<td>967.3±1.0</td>
</tr>
<tr>
<td>5</td>
<td>Phenylactic</td>
<td>4.1±0.1</td>
<td>2.2±0.1</td>
<td>3.2±0.1</td>
</tr>
<tr>
<td>6</td>
<td>Salicylic</td>
<td>10.3±0.2</td>
<td>8.7±0.2</td>
<td>9.7±0.2</td>
</tr>
<tr>
<td>7</td>
<td>Malic</td>
<td>1644.9±0.8</td>
<td>1513.0±0.7</td>
<td>1496.7±0.8</td>
</tr>
<tr>
<td>8</td>
<td>Citric</td>
<td>940.7±0.8</td>
<td>813.0±0.7</td>
<td>777.7±0.6</td>
</tr>
<tr>
<td>9</td>
<td>Vanillic</td>
<td>8.2±0.2</td>
<td>9.4±0.2</td>
<td>11.5±0.4</td>
</tr>
<tr>
<td>10</td>
<td>Palmitoleic</td>
<td>30.3±0.2</td>
<td>35.3±0.3</td>
<td>33.3±0.2</td>
</tr>
<tr>
<td>11</td>
<td>Stearic</td>
<td>31.4±0.2</td>
<td>25.3±0.2</td>
<td>29.6±0.2</td>
</tr>
<tr>
<td>12</td>
<td>Palmitic</td>
<td>1135.0±1.2</td>
<td>1215.5±1.2</td>
<td>1285.7±1.5</td>
</tr>
<tr>
<td>13</td>
<td>Heptadecanoic</td>
<td>30.3±0.2</td>
<td>35.3±0.3</td>
<td>33.3±0.2</td>
</tr>
<tr>
<td>14</td>
<td>Oleic</td>
<td>17.2±0.2</td>
<td>20.5±0.1</td>
<td>23.5±0.2</td>
</tr>
<tr>
<td>15</td>
<td>Linoleic</td>
<td>31.4±0.2</td>
<td>25.3±0.2</td>
<td>29.6±0.2</td>
</tr>
<tr>
<td>16</td>
<td>Linolenic</td>
<td>1047.1±1.0</td>
<td>1084.1±1.1</td>
<td>1139.1±1.3</td>
</tr>
<tr>
<td>17</td>
<td>Arachinic</td>
<td>13.1±0.1</td>
<td>14.3±0.2</td>
<td>16.8±0.1</td>
</tr>
<tr>
<td>18</td>
<td>Behenic</td>
<td>11.1±0.1</td>
<td>15.6±0.3</td>
<td>16.8±0.1</td>
</tr>
<tr>
<td>19</td>
<td>Tetracosanoic</td>
<td>22.1±0.2</td>
<td>27.4±0.2</td>
<td>31.8±0.2</td>
</tr>
<tr>
<td>20</td>
<td>Pentacosanoic</td>
<td>14.2±0.1</td>
<td>16.4±0.1</td>
<td>19.4±0.2</td>
</tr>
</tbody>
</table>

The qualitative composition of organic, including fatty acids, in plant raw materials of 3 species of Arctium is the same: 11 organics and 12 fatty acids (5 – unsaturated and 7 – saturated). The quantitative content of the identified acids is comparable, and the plant raw materials differed slightly in terms of this indicator. The highest content of the sum of identified organic acids is characteristic for the roots of A. minus and A. tomentosum. The content of malic, succinic, and citric acids is dominated in the roots of three species of Arctium, while the highest content of these acids is in the roots of A. minus. In the plant raw materials of three representatives of the genus is observed. The following pattern content of the malic and citric acid: A. minus>A. tomentosum>A. lappa. The regularity of succinic acid content is different: A. minus>A. lappa>A. tomentosum. Phenylacetic acid accumulated in the smallest amount of plant raw materials of the studied species.

The quantitative content of identified unsaturated fatty acids in the studied objects was 3 times higher than the content of saturated fatty acids. Of the unsaturated fatty acids, the content of linoleic and linolenic acids dominated and linoleic acid was twice as much as linolenic acid in the roots of three species of Arctium. A comparison of the results of determining the content of fatty acids in the roots of A. minus and A. tomentosum with the root of A. lappa showed that the variation in the content of each component between plant species is insignificant. For example, from the identified saturated fatty acids in the plant raw material, the highest content was for palmitic acid, which in the root of A. lappa was 1285.7±1.5 μg/g, while in the roots of A. minus and A. tomentosum – 1135.0±1.2 μg/g and 1215.5±1.2 μg/g, respectively. Roots of A. minus, A. tomentosum and A. lappa have the smallest content of pentacosanoic acid: 3.5±0.1 μg/g, 5.8±0.1 μg/g and 7.1±0.1 μg/g, respectively.

The results of determining the amount of content of the main groups of biologically active compounds in the roots of A. minus and A. tomentosum compared to the root A. lappa are given in Table 3.

As evidenced by the data in Table 3, the quantitative content of the amount of organic acids, polysaccharides, total polyphenols and amount of hydroxycinnamic acids in the roots of A. minus and A. tomentosum was slightly differed and was a little higher than it was in the root of A. lappa. The content of ascorbic acid in the plant raw materials of three species of Arctium was the same.
Quantitative determination of main groups of biological active compounds in the roots of *A. minus* and *A. tomentosum* compared to the root *A. lappa* (%.* n=5*, in terms of absolutely dry plant raw materials)

<table>
<thead>
<tr>
<th>Names of biologically active compounds</th>
<th>Quantitative content in the root of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. minus</em></td>
</tr>
<tr>
<td>Amount of organic acids (in terms of malic acid)</td>
<td>1.95±0.06</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Polysaccharides</td>
<td>5.55±0.50</td>
</tr>
<tr>
<td>Total polyphenols (in terms of pyrogallol)</td>
<td>4.41±0.10</td>
</tr>
<tr>
<td>Amount of hydroxycinnamic acids (in terms of chlorogenic acid)</td>
<td>2.44±0.05</td>
</tr>
</tbody>
</table>

### 5. Discussion of research results

According to the «Community herbal monograph on *Arctium lappa*, radix» and monograph «Burdock Roots» of SPhU 2.1, as a plant raw material «Arctii radix» can be used as root of *A. lappa*, *A. minus* and *A. tomentosum* [17, 18] while we did not find any information in the literature about a systematic comparative pharmacognostical study of the plant raw materials of these species of Arctium, which was done by us for the first time. Arctium is a dioecious herbaceous plant that forms a basal rosette of leaves in the first year and a stem with generative organs in the second. Medicinal plant raw materials are roots, which are harvested in the first year of life at the end of the growing season or at the beginning of the second year when the first leaves appear. Therefore, when harvesting raw plant materials, the identification of the plant species can be carried out only by the morphological features of the leaf plate of the basal rosette of roots.

Sokol O. V., in her work, described the morphological differences of the leaf blade *A. lappa*, *A. minus* and *A. tomentosum*: a heart-shaped form of a leaf with a blunt apex and a heart-shaped base in *A. lappa* and *A. tomentosum* and egg-shaped form of a leaf with a sharp top and a notched base in *A. minus* [2]. According to these signs, it is still possible to distinguish *A. minus* from the other two types of Arctium, but it is impossible to distinguish *A. lappa* from *A. tomentosum*. At the same time, the author also established a number of biometric indicators of plants in the first year of life, the results of which, for the studied plant species, are close in value and cannot be used as a key for species identification. The author noted differences in the morphometric indicators of the root system Arctium species of the first year of life in the morphological description: the root of *A. lappa* had the highest indicators of root neck width (3.3±0.1 cm), length (23.6±0.5 cm) and mass (174.0±6.5 cm) [2], which in our opinion is not significant when it is a medicinal plant raw material.

The plant raw material – burdock root can be purchased both in a pharmacy and outside the pharmacy network, and as a rule, the plant raw material is already crushed. Therefore, it is impossible to determine the above-mentioned morphometric indicators. In addition, when harvesting plant raw materials, the root neck is not harvested. Also, when harvesting root, we paid attention to the fact that the size, weight and branching of it depend on the size of the basal rosette: the larger the above-ground mass, the larger the root, and very often, when harvesting it, it is quite impossible to dig the underground organ (in plants with a large above-ground mass), it is refracted. These indicators are related not so much to the type of Arctium but more to the ecological conditions of plant growth.

In the morphological description of the root (fresh and dry) *A. minus* and *A. tomentosum* are described the following parameters: general appearance, nature of branching, shape, nature and colour of the surface and fracture, characteristics of the cortex, cambium and central part. When comparing the morphological features of the root of *A. lappa*, which is given in the monograph of Ph. Eur. 11.3 «Burdock root» [19], with descriptions of the signs of the roots of *A. minus* and *A. tomentosum* has some differences. In the monograph of Ph. Eur. 11.3, there is a description of the root neck of *A. lappa* (if it is available) and information that pieces of the root may have a longitudinal split into 3–4 parts. In Ukraine, burdock root is sold in crushed form because if you dry whole plant raw materials or pieces of 5–20 cm (as in the monograph), then after drying, the root becomes very strong, which greatly complicates the grinding process or makes it impossible. We did not record any splitting of the plant raw material when harvesting the root of 3 species of Arctium in large pieces. In this regard, this indicator is not significant for the roots of *A. lappa*, *A. minus* and *A. tomentosum*.

Comparing the results of the morphological description of the roots of *A. minus* and *A. tomentosum* with our previous results of the description of the roots of *A. lappa*, a distinctive feature is the presence of a visible ring of secretory channels (containers) in the root of *A. lappa*. In the monograph of SPhU 2.1 «Burdock Roots» it is stated: «on a section of the root along the line of the cambium, tissue rupture is visible» [18], which was not found in our samples which were studied. We also described the shape of the roots, which is not mentioned in the monograph of SPhU 2.1.

The anatomical structure of the roots of the first year of life of *A. lappa* was well studied, including by us [26], in contrast to the structure of the roots of *A. minus* and *A. tomentosum*, which was done for the first time. When comparing our results of studying the anatomical structure of the roots of *A. minus* and *A. tomentosum* with the structure of the roots *A. lappa*, which was studied earlier [26], one clear distinguishing feature can be distinguished at the species level: the shape of the receptacles of the schizogenic type. At the root of *A. lappa*, they are rhombic (the corners of the rhombus are not elongated) [26], frequent and filled with brown contents,
while at the root of *A. minus*, they are round and thin, and at the root of *A. tomentosum* - rhombic (elongated corners). Brown content very rarely present in root receptacles of *A. minus* and *A. tomentosum*. Also, it can be a small cavity in the centre roots of *A. minus*. More differences in the anatomical structure of the roots of the 3 species of Arctium were not found. When comparing our description of the anatomical features of the root of *A. lappa* with its description in the monograph of Ph. Eur. 11.3 [19] a type of vessels was different: in the monograph of Ph. Eur. 11.3, reticulate vessels are indicated, in our samples plant raw material of *A. lappa* and in the monograph SPhU 2.1 root has ladder vessels [18]. Also for the roots of *A. minus* and *A. tomentosum* characteristic ladder vessels.

In the literature available to us, we did not find information about the anatomical structure of the root of *A. minus*. There is information about the anatomical structure of the root of the second year of life *A. tomentosum* and *A. lappa*, which was studied within the framework of the anatomical study of the roots of plants of the tribe Cardueae of the Asteraceae family which are used in medicine [27]. The authors studied the anatomical structure of the roots of the second year of life and established that the cortex and endodermal resin ducts lost in the course of rhytidome formation, the phelemma is wide, formed by cells with thickened membranes; the secondary phloem is wide (in some roots it may contain groups of fibers), but not wider than wood; secondary xylem is mainly represented by radially located reticular vessels [27]. At the same time, the authors do not indicate distinguishing features between the roots of the second year of *A. lappa* and *A. tomentosum* and according to their data, the vessels are reticulated in these roots. In addition, the authors do not provide information about the reserve substance that is characteristic of the roots of Arctium species - inulin. Perhaps the identification of inulin was not part of their work tasks.

In recent years, a significant number of scientists have devoted their work to the study of the chemical composition and pharmacological activity of the root, leaves and fruits of *A. lappa* with the dominance of studies on leaves and fruits [28–31]. Information on studying the root *A. minus* and *A. tomentosum* is insignificant. Author Skowronska W. with colleagues from the root and aerial part of *A. lappa* and *A. tomentosum* received extracts (the conditions of obtaining and the extractant are not specified) and conducted a comparative analysis of the quantitative content and chemical profile of polyphenols and anti-inflammatory activity (by the effect on lipooxygenase activity) and antioxidant activity [32]. As a result of such a comprehensive comparative study of root extracts of *A. lappa* and *A. tomentosum* the authors say that these extracts had significant differences both in the chemical composition and in the quantitative content of the total polyphenols and in the manifestation of biological activity. Therefore, the roots of *A. lappa* and *A. tomentosum* should be used separately and in their opinion, the plant raw materials of these types of plants cannot be interchanged [32].

The results of our comparative study show that the component composition of organic acids, including fatty acids, is the same. The quantitative content of the amount of organic acids, polysaccharides, total polyphenols and the amount of hydroxycinnamic acids is comparable.

In our previous works, the quantitative content of the amount of organic acids, ascorbic acid, polysaccharides, and the amount of hydroxycinnamic acids in the roots of *A. lappa* was determined [26]. But for this, other methods of determination were used. We determined the quantitative content of the main groups of biologically active compounds using modern methods of the pharmacopoeial variety for a systematic comparative analysis of the roots of 3 Arctium species and establishing the possibility of interchangeability of plant raw materials. As a result, it was found that the biggest difference is observed in the quantitative content of the amount of organic acids: in the root of *A. lappa*, it is the lowest and does not reach 1.5 %, while at the root of *A. minus* and *A. tomentosum* is higher and comparable – about 2 %. At the same time, the amount of identified organic acids (excluding fatty acids) was the highest in the root of *A. lappa* and lowest at the root of *A. tomentosum*.

The polysaccharide content of the root of *A. lappa* was determined earlier and was 4.45±0.20 % [26]. Comparing the results of the content of polysaccharides in the roots of *A. minus* and *A. tomentosum* with the root *A. lappa*, fluctuations within 1 absolute % are observed. According to monographs Ph. Eur. 11.3 and SPhU 2.1, Arctium root is standardized by the content of the number of hydroxycinnamic acids, which should be at least 2 %. We determined the content of this group of substances in the roots of 3 Arctium species. The results showed that all the studied plant raw materials met the requirements of SPhU 2.1 (more than 2 %), and the quantitative content of the amount of hydroxycinnamic acids is comparable and slightly higher in the roots of *A. minus* and *A. tomentosum* than in the roots of *A. lappa*. Similar results are inherent in the quantitative content of the amount of polyphenols in the plant raw materials.

Studies of 3 species of the Arctium genus conducted at this stage showed a close component composition of acids and quantitative content of certain groups of substances, which confirms the prospects of further in-depth comparative pharmacognostic research of the plant raw materials of these species of Arctium.

**Practical Relevance.** The results experimentally confirm that when harvesting wild plant raw materials – «Arctii root» of the first year, it is impossible to identify by morphological features the plant raw materials of each of the studied Arctium species, since the distinguishing features are insignificant and they are not diagnostic. At that time, the diagnostic feature of the anatomical structure (the shape of the schizogenous receptacles) is also of little significance at the species level. The results of studying component composition and determination of the quantitative content of a num-
ber of major groups of biologically active compounds showed their comparability with minor differences, which, in our opinion, are not significant.

The aim of our work was not a comparative study of the component composition of organic, including fatty acids, and quantitative content some groups of biological active compounds which was studied components in plant raw materials from different places and years of harvesting, because we started from the monograph SPhU 2.1 in which, most likely, the developers took into account the years and places of harvesting.

According to our observations in connection with climate changes in some regions of Ukraine *A. lappa* one is reducing its areas, and the more widespread is *A. tomentosum*, which was one of the reasons for our comparative study. The results of our research indicate that, from the point of view of sources of plant raw materials, it is necessary to return to monograph SPhU 2.1.

**Research limitations.** The limitation of the research is the comparative study of the component composition of only organic, including fatty acids. It is promising to study the comparative component composition of phenolic nature compounds and biological activity of plant raw materials, which are harvested from geographically wider regions and different years, possibly in comparison with foreign samples.

**Prospects for further research.** The prospect of further research is to continue an in-depth comparative study of the roots of the 3 Arctium species in order to more thoroughly confirm or deny their interchangeability.

### 6. Conclusions

1. For the first time it was carried out a comparative pharmacognostical study of the roots of *A. tomentosum* and *A. minus* in comparison with the root of *A. lappa* which showed minor differences between the roots of 3 Arctium species and confirms their interchangeability at this stage, especially when harvesting wild plant raw materials, when identification of the species at the botanical level is impossible.

2. For the first time, a comparative morphological and anatomical study of the roots of *A. tomentosum* and *A. minus* in comparison with the root of *A. lappa* was carried out. As a result, a distinctive microscopic feature of the roots was established: the shape of the receptacles of the schizogen type.

3. For the first time it was determined the component composition of organic, including fatty, acids for the roots of *A. tomentosum* and *A. minus* in comparison with the root of *A. lappa* which is the same. The content of 11 organics and 12 fatty acids in plant raw materials of 3 Arctium species was identified and determined.

4. The quantitative content of amount of organic acids, ascorbic acid, polysaccharides, total polyphenols, and amount of hydroxycinnamic acids in the roots of *A. tomentosum* and *A. minus* in comparison with the root of *A. lappa* and it was established that these indicators are comparable which confirms the possibility of using the roots of all 3 Arctium species as medicine plant raw material.

5. The obtained research results will be used in the further study of the chemical composition and biological activity of plant raw materials of Arctium species and phytoremedies from it.

### Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

### References

1. Genus Arctium L. Who plant list. Available at: https://wfoplantlist.org/taxon/wfo-4000002920-2020-12?page=1


19. European Pharmacopoeia 11 ed. Supplement 11.3. Available at: https://pheur.edqm.eu/home

20. Derzhavna farmakopeia Ukrainy Dop. 7.2 (2.0). (2024). Kharkiv: Derzhavne pidpryiemstvo «Ukrainskyi naukovyi farmakopeinyi tsentr yakosti likarskykh zasobiv».


32. Skowrońska, W., Granica, S., Dziedzic, M., Kurkowiak, J., Ziaja, M., Bazylik, A. (2021). Arctium lappa and Arctium tomentosum, Sources of Arctii radix: Comparison of Anti-Lipoxgenase and Antioxidant Activity as well as the Chemical Composition of Extracts from Aerial Parts and from Roots. Plants, 10 (1), 78. https://doi.org/10.3390/plants10010078

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