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Research Article

Comparative Analysis of the Content of Sum of Hydroxycinnamic Acids from Leaves of *Actinidia arguta* Lindl. Collected in Ukraine and China

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Leaves of *Actinidia arguta* Lindl. (*A. arguta*) are a promising raw material for pharmaceutical production. *Actinidia* is cultivated in Ukraine, and its homeland is China, so raw materials may have different origins. Hydroxycinnamic acids (HCAs) are one of the important biologically active substances of *A. arguta* leaves, which provide the pharmacological action of this raw material. The aim of the study was to identify and compare the quantitative content of HCAs in the leaves of *A. arguta* harvested in Ukraine and China in different phases of the growing season. Microscopic and phytochemical studies of the leaves of *A. arguta* are conducted. After histochemical reaction with the nitrite-molybdenum reagent, idioblast cells with HCAs in *A. arguta* leaves are stained on brick-red color. The amount of the sum of HCAs was determined by absorption UV-spectrophotometry in terms of rosmarinic acid at wavelength 505 nm after reaction with a nitrite-molybdenum reagent. It was found that the *A. arguta* leaves contain high levels of HCAs (to 2.69%). The highest HCAs level was recorded in July, which was decreased somewhat in August. Histochemical reactions for the detection of HCAs in fresh *A. arguta* leaves can be used to identify plants of the genus *Actinidia* Lindl., which are potential sources of HCAs. The content of HCAs is independent of the region of plant growth, but its quantity varies during the growing season. So, during July, the leaves can be collected from male plants, and during the end of August and start of September, leaves can be collected from both male and female plants. This indicates the prospect of using the leaves of *A. arguta* as a source of raw materials for pharmacy and medicine.

1. Introduction

The search for plant sources of biologically active substances includes the study of the influence of environmental factors from different places of plant growth on the content of compounds with medicinal potential. Plant growth conditions in different longitudes and latitudes are markedly different [1]. Various environmental limiting factors, such as ambient temperature, carbon dioxide levels, lighting, ozone, groundwater status, salinity, and soil fertility, have a significant impact on biochemical reactions in medicinal plants [2]. Plants can synthesize a variety of secondary metabolites to cope with the negative effects of stress. Secondary plant metabolites are a group of natural classes of compounds that are synthesized by various biochemical pathways. The content of these substances in plants is highly dependent on environmental influences [3]. The same plant species that grow in different environmental conditions have significant differences in the formation and accumulation of primary and secondary metabolites [3–8].

Secondary metabolites are used as important natural remedies with a wide range of pharmacological activities. One of the important groups of secondary metabolites are phenolic compounds, which perform many physiological functions for plant survival and are fundamental in the adaptation of plants to changes in the environment [9]. Hydroxycinnamic acids, which belong to the group of phenolic compounds, are an important group of secondary metabolites of plants that are involved in many important plant functions. For hydroxycinnamic acids, the directions of pharmacological action as anti-inflammatory, antiallergic, antiplatelet, antitumor, detoxifying, hepatoprotective, bactericidal, and antiviral is established [10–15].

Variations in the synthesis of plant metabolites often depend on growing conditions. Therefore, the study of differences in the quantitative content of secondary metabolites, in particular, hydroxycinnamic acids, in plants from different places of growth is essential for understanding the characteristics of plants of the same species, whose raw materials are harvested in different regions.

In the manufacture of medicines, the quality of the final product depends on the quality of the raw materials. The content of biologically active substances in plants is influenced by environmental conditions. Therefore, to effectively develop methods for standardizing plant raw materials for the content of secondary metabolites, it is important to take into account the different geographical origins of plant.

A promising source of hydroxycinnamic acids for pharmacy and medicine are the leaves of plants from the genus *Actinidia* Lindl. [16–18], which require more detailed phytochemical studies to develop methods of standardization.

Actinidia arguta Lindl. (*A. arguta*), named as kiwiberry, is distributed in the eastern and northern parts of China, Korea, Japan, Sakhalin, and the Kuril Islands cultivated in various regions of Asia, Europe, North America, New Zealand, and Australia. The northern limit of growth of *A. arguta* runs along 46°40′ north latitude. Due to its frost resistance, *A. arguta* is introduced into horticulture in Canada, Chile, France, and the United States. The largest *Actinidia* plantations are concentrated in the United States (Oregon). Frost-resistant varieties of *A. arguta* are also successfully cultivated in Poland [19].

The Global Biodiversity Database (https://www.discoverlife.org) contains an interactive map that includes registered wildlife habitats of *A. arguta* in the world, as well as marked botanical gardens where it is successfully cultivated (Figure 1). To obtain complete information about the indicated places of growth of *A. arguta*, you need to select the marked points on the map.

The homeland of kiwiberry is China, but it is well cultivated in Ukraine and has a high yield. Therefore, in Ukraine, kiwiberry is becoming increasingly popular in home gardens and industrial plantations. Researchers of the M. M. Grishko National Botanical Garden have for many years created the largest collection of plants of the genus *Actinidia* Lindl. in Ukraine, which includes not only well-researched and popular species but also new varieties and forms [20].

The vegetative mass of leaves in *Actinidia* vines is quite large and contains valuable biologically active substances. Therefore, it is important to study the presence and quantitative content of such phenolic compounds, such as hydroxycinnamic acids in *A. arguta* leaves cultivated in Ukraine, in comparison with raw materials harvested from wild plants of *A. arguta* in China to expand the raw material base for the development and creation of new herbal medicines.

2. Materials and Methods

2.1. Samples. The research study was conducted based on the Department of Pharmacognosy and Botany of the Bogomolets National Medical University (2020–2021).

The object of the study was generatively mature plants of kiwiberry (*A. arguta*), grown under different soil and climatic conditions, namely, from cultivated plants in Ukraine (Kyiv city, M. M. Grishko National Botanical Garden) on gray forest soil and from wild-growing plants in China (Jiamusi University, Heilongjiang province) on black earth. As a material for investigation, we used leaves of fifteen-year-old plants of *A. arguta*, which grew in the collection of M. M. Grishko National Botanical Garden (Ukraine) and in Jiamusi (China) during summer. Leaves samples of female and male plants were collected during June-September (Figure 2(a)). Leaf samples were dried by air-shadow drying (Figure 2(b)).

The male and female *A. arguta* leaves get into the plant raw material during the leaves collection from wild-growing plants in China. More leaves from female plants will be harvested as plant raw material in Ukraine because 5–7 female plants are planted next to one male plant during *A. arguta* cultivation. It means that leaves, as raw material will come more from female plants. Therefore, it is important to compare the HCAs content in female leaves collected in Ukraine and China. The harvesting time affected to the quantitative content of different BAS and to the HCAs also. We collected male and female *A. arguta* leaves in different months for a comparative determination of HCAs content.

Cultivated male plants of *A. arguta* can also become raw materials to expand the raw material base of medicinal plants. The content of HCAs in *A. arguta* leaves from male plants cultivated in Ukraine was also studied.

Jiamusi city is located in the eastern part of the northernmost province of China, Heilongjiang. The city is in a moderately cold zone with a humid continental climate. The average rainfall is 627 millimeters. The soils are mostly black soil with high humus content.

The climate of the area in which Kyiv is located is moderately continental, with an average annual temperature 9.4°C. M. M. Grishko National Botanical Garden, which located in Kyiv (N, 50°27′; E, 30°31′), is one of the biggest scientific and practical Ukrainian centers of introduction and acclimatization of nontraditional plants, selection and spreading of new cultivars, which may be successfully cultivated on commercial plantations and in private gardens.

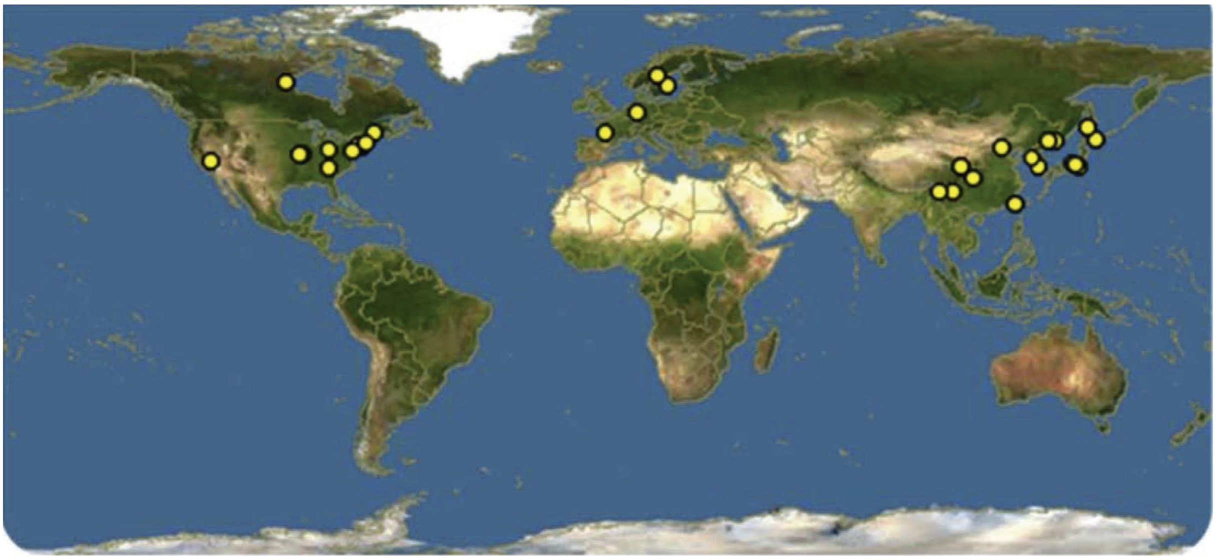


Figure 1: Distribution map of *Actinidia arguta* Lindl. in the world by Global Biodiversity Information Facility Database (https://www.discoverlife.org).

(a) (b)

Figure 2: Leaves of female plant *A. arguta* (a) freshly harvested and (b) dried collected in Ukraine.

The garden is located on the border of two climatic zones—wooded district and forest steppe in the southeastern part of city on the slopes of the Pechersk hills near the Dnipro River. The main type of soil is dark gray podzolic. Due to the crossing of locality, the garden soil is rather washed out and characterized by low humus content. The climate of the area is continental with an average annual temperature of 7.6°C; the average temperature in January is −5.5°C, in June −20.4°C. The winter in Kyiv is softened by the periodic invasion of Atlantic air masses. The frost-free period in Kyiv is 165–180 days on average. The average annual amount of precipitation in Kyiv is 550–650 mm, and the relative humidity is 73–76%. The duration of the period without frost, temperature conditions, and rainfall during the active growing season create a suitable possibility for the successful cultivation of different fruit and berry plants from regions with similar and sometimes more mild climatic conditions.

2.2. Determination of HCAs *in the A. arguta Leaves by Chemical and Histochemical Reactions.* Chemical reaction by using nitrite-molybdenum reagent as the Arnov test was performed as described by Gawlik-Dziki et al. [21].

2.2.1. Preparation of Extracts for the Detection of Hydroxycinnamic Acids. 3.00 g of crushed *A. arguta* leaves (separately from males and females) were filled with 50% alcohol. Extracted in a water bath in a flask under reflux for 20 minutes, the extract was cooled, and after cooling, it was filtered.

2.2.2. Preparation of Nitrite-Molybdenum Reagent. Reagent was prepared by dissolving 10% (m/v) sodium molybdate and 10% (m/v) sodium nitrite in distilled water. To prepare a dilute sodium hydroxide solution, 8.3 g of sodium hydroxide was made up to 100 ml with purified water in a volumetric flask. All reagents were prepared daily.

2.2.3. Reaction with Nitrite-Molybdenum Reagent. To 1 ml of each extract, 2 ml of 0.5 M hydrochloric acid solution, 2 ml of a mixture of 10% sodium molybdate solution and 10% sodium nitrite solution, and 2 ml of dilute sodium hydroxide solution were added in the specified order. The formed reaction products were brick-cherry in color.

The investigation of the character of the localization of HCAs in the *A. arguta* leaves was carried out by light microscopy after reaction on cross sections of petiole and central vein of the leaf with nitrite-molybdenum reagent [22]. Temporary micropreparations were examined in the light trinocular microscope XSP-146T of ULAB at a magnification of 40, 100, 400, and 1000 times. Snapshots were taken using the camera Canon EOS 550 DSLR.

2.3. Comparative Analysis of HCAs Content in the A. arguta Leaves by Spectrophotometry. The quantity of HCAs sum was defined by absorption UV-spectrophotometry in amounts equivalent to rosmarinic acid at wavelength 505 nm after reaction with the nitrite-molybdenum reagent. As a standard for comparison, rosmarinic acid was used, since after the reaction with a nitrite-molybdenum reagent, extracts from the studied types of raw materials form a maximum absorption at 505 nm, as well as rosmarinic acid (Figure 3). HCAs give maxima of absorption of the reaction product with the nitrite-molybdenum reagent in the wavelength range from 490 to 527 nm. In the aqueous alcoholic extract of *A. arguta* leaves, the sum of HCAs forms the maximum absorption with nitrite-molybdenum reagent at a wavelength of 493 nm. Therefore, we have chosen a unified method for calculating the content of the sum of HCAs in terms of rosmarinic acid at a wavelength of 505 nm, which is given in the monograph State Pharmacopeia of Ukraine 2.0 Java tea.

The quantitative determination of the HCAs sum in the investigated types of raw materials was carried out according to the method of quantitative determination of HCAs amount in Java tea described by the State Pharmacopeia of Ukraine 2.0 [23].

The statistical analysis of the results was carried out in accordance with the monograph of the State Pharmacopoeia of Ukraine 2.0 "Statistical analysis of the results of a chemical experiment" using Microsoft Excel 2010 for Windows.

3. Results

3.1. The Results of Determination of HCAs in the A. arguta Leaves by Chemical and Histochemical Reactions. As a result of the reaction of alcoholic extracts from the leaves of male and female plants of A. arguta with nitrite-molybdenum reagent, the formation of brick-cherry color of the formed reaction products was observed (Figure 4).

Histochemical reactions on cross sections of the petiole and central vein of the leaf with the nitrite-molybdenum reagent showed the presence and character of the localization of HCAs in *A. arguta* leaves.

In the study of the localization of HCAs in the studied leaves of female and male plants of *A. arguta*, we observed the formation of brick-red color in idioblast cells in the xylem area, phloem, as well as in the parenchyma under the integument. Figure 5 shows the result of histochemical reaction on the leaves of females and males of *A. arguta* with the nitrite-molybdenum reagent.

The result of our reaction was stable. The color did not disappear quickly over time. Conducted histochemical reactions provide additional information in establishing the identity of medicinal plant raw materials. They revealed the localization of the studied group of substances directly in tissues and cells.

3.2. Analysis of the Content of HCAs Collected in Different Regions and Different Months. The content of HCAs sum is in the range from $1.51 \pm 0.04\%$ to $2.69 \pm 0.13\%$ (Figure 6). There are different medicinal plant materials in Ukraine, which have standardized content of HCAs detected with spectrophotometry. For example, *Echinacea purpurea* radix must content not less than 2.0% HCAs in amounts equivalent to chicoric acid and dry raw material. *A. arguta* leaves contain almost the same amount of HCAs.

The HCA content in *A. arguta* leaves is independent of plant growth conditions. The leaves collected in different regions of China and Ukraine contained almost the same HCAs sum, although it was slightly higher in leaves mass collected in Jiamusi (Figure 6). Samples of leaves from China, which have a slightly higher content of HCAs, were collected from wild plants that grow in their natural range. It was found that the content of HCAs in cultivated plants on the territory of Ukraine is slightly lower than in wild plants of *A. arguta* from China. The maximum content of HCAs is observed in July, and in August it reduces slightly. It was determined that the content of HCAs decreases during the vegetation until the end of the summer in the same way in *A. arguta* leaves harvested in Jiamusi and in Kyiv. Therefore, it is advisable to harvest the leaves of *A. arguta* in July to obtain the raw materials with the highest content of HCAs for the next research study of pharmacological action. At this time, you can use leaves from the shoots of female *A. arguta* plants, the main function of which is to provide the plants with pollen during their flowering period.

3.3. Analysis of HCAs Content in the Leaves of Female and Male Plants. It should be noted that the leaves of female and male plants differ in their HCA content (Figure 7). Thus, in June, the leaves of female plants accumulated more HCAs ($2.26 \pm 0.10\%$) compared to male ones ($1.67 \pm 0.08\%$). In July, their contents were leveled, and in the following months of observation, there is a steady tendency of decrease in the level of acids in female plants that may be associated with the onset of the period of ripening of the fruit. Therefore, the collection of *Actinidia* leaves of male plants is recommended in July and August. At this time, the leaves of the shoots of female *A. arguta* plants, which are removed during the summer pruning, may be used. Raw materials can also be harvested from female plants in August because the content of HCAs remains relatively high ($1.51 \pm 0.04\%$). In September, after the harvest, you can collect leaves from all plants, regardless of sex, because the content of HCAs does not fall critically ($1.13 \pm 0.05\%$ in female and $1.85 \pm 0.09\%$ in male plants).

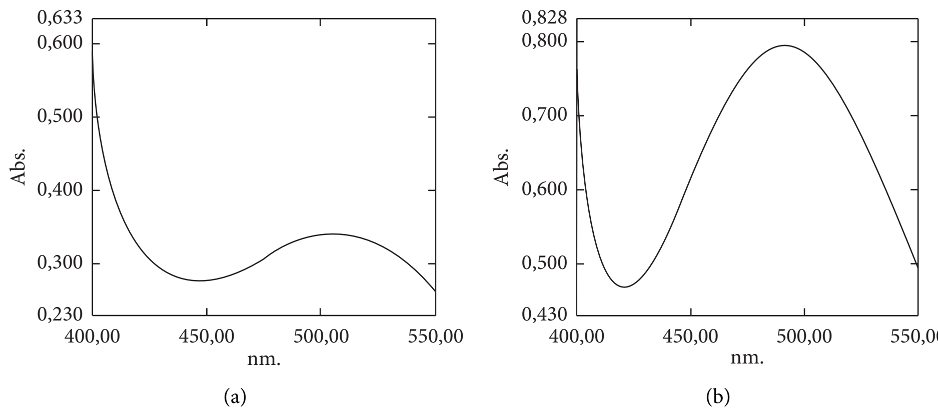


Figure 3: Electronic spectrum absorption of alcoholic solution of rosmarinic acid with the nitrite-molybdenum reagent (a). Electronic spectrum absorption of alcohol-aqueous extract of *A. arguta* leaves with nitrite-molybdenum reagent (b).

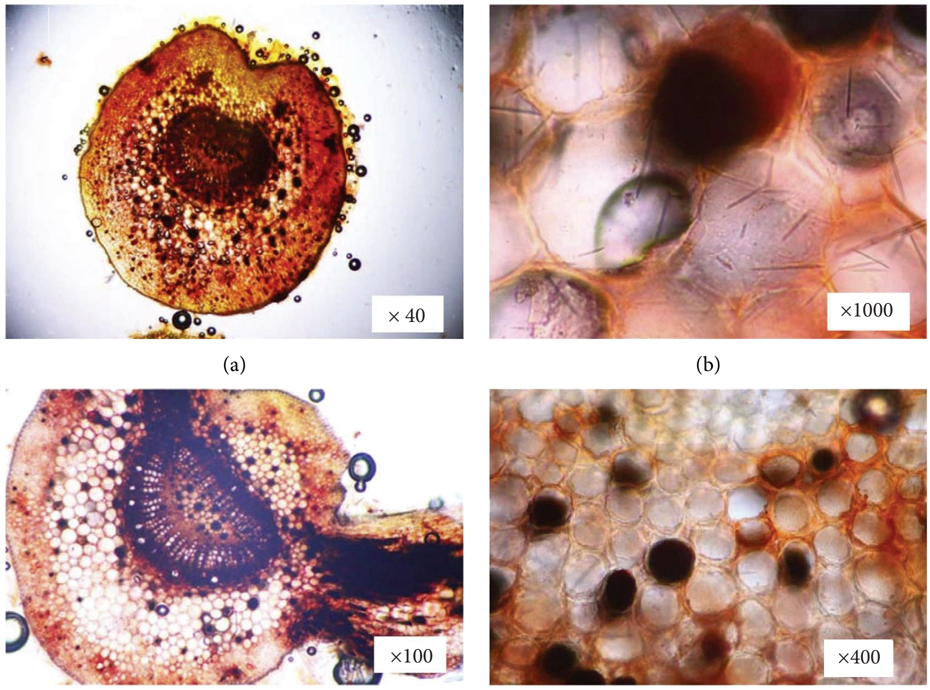
Figure 4: The result of the reaction with the detection of HCAs with an extract from the leaves of females of *A. arguta*.

4. Discussion

Chemical and histochemical reactions for the determination of HCAs in the leaves of *A. arguta* showed positive results and could be used for HCAs identification in this plant raw material. The reagents we have chosen are quite economical and easy to use. According to the saturation of the color of the reaction products and the number of idioblast cells in the section, it can be assumed that hydroxycinnamic acids contain approximately the same amount in the leaves of male and female individuals of *A. arguta*. The obtained results can be used as an auxiliary indicator to establish the identity of raw materials in the development of quality control method "*Actinidia* leaves."

The spectrophotometric pharmacopoeia method allows for the maximum absorption of the reaction products with the nitrite-molybdenum reagent to determine the sum of all HCAs in the raw material. We determined the content of HCAs in *A. arguta* leaves harvested from female and male plants during the whole vegetation period by the method of spectrophotometric research. In June, there is a fairly high content of HCAs, but for the plant harvest in this period is not useful, because the vegetative mass of leaves has yet to perform its assimilative function. In addition, young leaves harvested in June turn black quickly during drying. This indicates active enzymatic biochemical processes in the leaves during this period. In June, the leaves of male plants synthesize the least HCAs for the entire growing season ($1.67 \pm 0.08\%$). In the first two summer months, more HCAs accumulated in the leaves of female plants ($2.26 \pm 0.10\%$ in June and $2.37 \pm 0.12\%$ in July). In August and September, their content decreased compared to the content in July by 37% and 52%, respectively. This decrease in the content of HCAs in the leaves of female plants can be associated with the beginning of the process of fruit ripening. All biochemical processes involved in the formation of biologically active substances are concentrated during this period in fruits. In leaves harvested from male plants, the content of HCAs is highest in July ($2.20 \pm 0.11\%$), and in August their content decreases by only 8.6%. The results showed that the best period for collecting leaves to create potential drugs is the end of July–August because it was then that the spectrophotometric method revealed the highest amount of HCAs for rosemary acid (2.0% on average).

There is a positive correlation between the amount of HCAs in plants growing in Ukraine and China. Our research has shown that *A. arguta* leaves collected from cultivated plants in Ukraine have a slightly lower HCA content compared to wild plants collected in the natural growth zone in China. Thus, leaves harvested in July in the province of Jiamusi accumulate only 12% more HCAs than *A. arguta* leaves harvested in July at research sites in the M. M. Grishko National Botanical Garden in the city of Kyiv, Ukraine. This gives reason to believe that the leaves of the cultivated plant *A. arguta* are a promising raw material base for research to develop new potential remedies.



(a) (b) (c) (d)

Figure 5: Locations of HCAs in the leaves of females (a, b) and males (c, d) of *A. arguta* by histochemical reaction with the nitrite-molybdenum reagent.

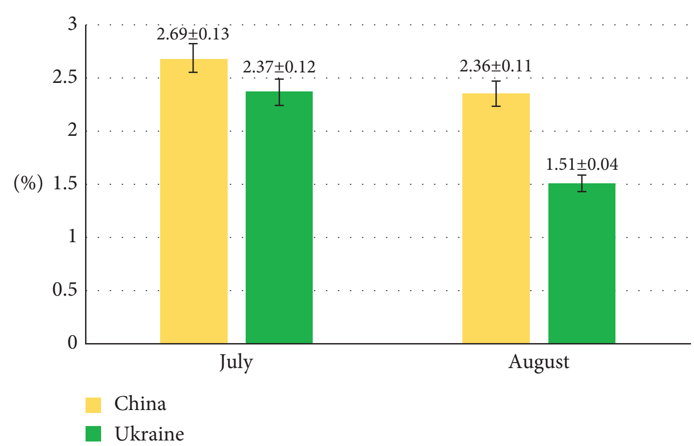


Figure 6: Content of the sum of HCAs in *A. arguta* leaves collected in Jiamusi (China) and Kyiv (Ukraine) (p-value < 0.05).

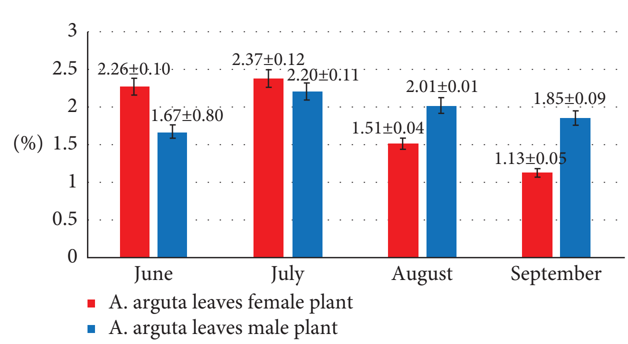


Figure 7: The content of the HCAs sum in *A. arguta* leaves collected in Kyiv (Ukraine) (p-value < 0.05).

5. Conclusions

A. arguta leaves contain a high level of HCAs (up to 2.0%), which is independent of the region of plant growth. Its quantity varies during the growing season, with the highest HCA content in July. During this period, the leaves of male plants, whose main function is to provide the process of pollination of female flowers during the flowering period in May, may be used to harvest raw materials. In August and in September, after harvesting fruits, the HCA content in *A. arguta* leaves decreases somewhat but remains rather high, so the leaf mass during this period may be harvested with both male and female plants. Therefore, the leaves of *A. arguta*, cultivated in Ukraine are a promising source for obtaining extracts with a high content of HCAs, which require future research on pharmacological activity. Certain features of the localization of hydroxycinnamic acids can be used to identify plant raw materials in the development of methods for quality control of raw materials.

We can note that *A. arguta* leaves collected in China have the higher content of HCAs. China is a homeland of *A. arguta*, and natural growth conditions contribute to the accumulation of secondary metabolites in plants.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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