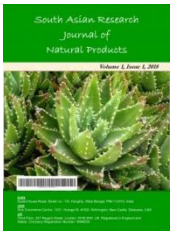

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Substantiation of the Rational Drying Conditions for the Herbal Raw Material of Goutweed (*Aegopodium podagraria* L.) Aerial Part

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NIP, OVT, VVE designed the study and wrote the protocol as well as managed the manuscript editing, improvement and completion. Author OVT harvested raw material. Author SIS identified and author NIP managed the drying process of the samples. Authors OVT, SIS and OOK managed the analyses of the study. Author OVT wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study aims to substantiate the rational conditions for the industrial drying of goutweed (*Aegopodium podagraria* L.) aerial part enabling its use in the pharmaceutical and food industries.
Methodology: The traditionally used shade drying (18–22°C for 3–5 days, sample 1) was compared with the industrial drying with the help of the original equipment presupposing mixed heat transfer (60–70°C for 90 min or 50°C for 120 min, sample 2 and sample 3, respectively). The moisture content (loss on drying) was determined, hydroxycinnamic acids and flavonoids were

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identified by HPLC, and the quantitative content of hydroxycinnamic acids was measured using direct spectrophotometry and spectrophotometry of the coloured complexes.
Results: It has been shown that the moisture content is comparable in all samples and there are no significant changes in the spectrum of biologically active substances identified by HPLC in the samples 2 and 3. The quantitative content of hydroxycinnamic acids is decreased in these samples that may be associated with the activation of polyphenol oxidase at the beginning of drying. Thus, the industrial drying methods do not cause principal changes in the spectrum of goutweed active substances and are advantageous in shortening the drying time. Nevertheless, the drying conditions modifications (presumably, an increase in the temperature at the beginning of drying) are needed for better preservation of hydroxycinnamic acids in goutweed raw material. The further studies will address these issues, since the shade drying, despite the possibility of obtaining of the final product with satisfactory quality (that is consistent with the previous data obtained under the laboratory conditions), is unacceptable for use in the manufacturing facilities.

Keywords: Aegopodium podagraria L.; herbal raw material; drying process; hydroxycinnamic acids.

1. INTRODUCTION

Nowadays the people all over the world are becoming more interested in herbal medicines. More than 80% of the world's population predominantly use traditional medicines for their primary health care needs [1], The patients in the developed countries also often turn to the traditional herbal medicines. Besides, all over the world there is an increase in the use of the functional foods with the beneficial health effects [2,3]. At the same time, safety and quality of herbal materials and products is of great importance and the technical guidelines related to quality assurance and control of herbal medicines are being developed by WHO [4]. In this context, there is a need for research in the field of medicinal plant drying. It is noted [5] that the ways of drying used in practice do not correspond to those recommended in the literature. Moreover, it is emphasized that each species has to be investigated individually and general recommendations are hardly possible.

Our efforts are focused on the phytochemical and pharmacological studies of *Aegopodium podagraria* L. (goutweed) belonging to the family *Apiaceae*. This medicinal plant is promising for the development of the drugs as well as functional foods or dietary supplements, that is substantiated by a long history of its use as a vegetable and a significant evidence of safety and efficacy (shown in the preclinical studies). This perennial plant is widespread within the temperate climate zones (including Europe, Siberia, the Caucasus, Kazakhstan and Central Asia mountainous regions). It has been naturalized in North America and Australia. Goutweed is ubiquitous and the raw material of its aerial part is available for manufacturing at respectively low cost, being affordable even for the developing countries. It is widely used in traditional medicine and consumed as vegetable and as fodder plant [6–11]. It has significant prospects for the use in food industry [10,12,13]. The recently obtained results have confirmed the valuable biological activity of goutweed including nephro- and hepatoprotective properties, the ability to normalize uric acid and glucose metabolism [13–18] that is pathogenetically important in the "diseases of civilization."

Hydroxycinnamic acids, flavonoids, coumarins, polyacetylene compounds, essential oil components, micro- and macroelements were identified in goutweed aerial part. The procedures of hydroxycinnamic acids quantitative assay in goutweed aerial part by spectrophotometric methods were developed [15,19]. Chromatographic (PC, TLC, HPLC) methods were proposed for the analysis of this raw material and preparations obtained from it. HPLC was applied for aerial part analysis and it allowed identifying ferulic and chlorogenic acids as well as trifolin and rutin. Chlorogenic acid was shown to be the predominant among hydroxycinnamic acids [15]. Capillary electrophoresis was employed in the fingerprint analysis of goutweed leaves and stems, as well as water and ethanol extracts [20].

Generally accepted methods were used for the drying of goutweed raw material in these works (for example, in the study [20], the samples were dried at room temperature and kept during two months). Drying at room temperature was also used in our previous studies [14,16-18]. Nevertheless, in these studies a limited quantity of raw material was processed under the laboratory conditions.

Optimization of the methods of goutweed raw material drying was considered in the work [12]. The proposed methods were as follows:

shade drying (25–30°C for 3–5 days, hygroscopic moisture content 15–20%, thickness of raw material layer 1–2 sm), using of dryers (convective drying, 30–35°C for 2 days, hygroscopic moisture content 12–15%, thickness of raw material layer 3–4 sm), using of microwave dryers (90–100°C for 7–10 seconds, hygroscopic moisture content 10–12%, thickness of raw material layer 3–4 sm. However, the specific feature of this work is the use of the rolling machine with the subsequent fermentation of the raw material with the appearance of copper colour. Proceeding from the significance of the phenolic compounds for goutweed biological activity [14,15,19,20], this approach can hardly be explained (moreover, this work has not addressed the changes in the phenolic compounds spectrum during the fermentation and the respective changes in the biological activity). Besides, the described methods are not widely available.

Quantitative analysis of hydroxycinnamic acids [19] has shown that May and June are the optimal term of goutweed harvesting in Ukraine and supposedly in the other habitats within the temperate zone. Since the raw material is available during the limited time period, its practical use in the manufacturing facilities is possible only after the preliminary preparation by the standardized methods (including drying). The absence of such methods substantiation limits the potential use of goutweed for functional foods and drugs development. At the same time, the available data do not elucidate whether the industrial drying methods are beneficial for preserving phenolic compounds in goutweed raw material. Hydroxycinnamic acids are of special interest in this context. Taking into account the necessity to investigate the drying conditions individually for each plant species [5], we aimed to compare the influence of the different drying conditions on the quality of goutweed aerial part raw material.

The objective of this study is to determine the rational conditions for the industrial drying of goutweed (*Aegopodium podagraria* L.) aerial part enabling its use in the pharmaceutical and food industries.

The tasks of the study were as follows:

1 to substantiate the choice of the method of drying of goutweed aerial part raw material;
2 to determine the influence of the chosen drying conditions on the moisture content in the final product (loss on drying);
3 to determine the influence of the chosen drying conditions on the qualitative profile of the biologically active substances (namely, hydroxycinnamic acids and flavonoids) identified by HPLC analysis;
4 to determine the influence of the chosen drying conditions on the concentration of the biologically active substances (namely, hydroxycinnamic acids) in the final product.

2. MATERIALS AND METHODS

2.1 Plant Material

The aerial parts of *Aegopodium podagraria* L. were collected from the natural population in May. Voucher specimens were identified by Ass. Prof. Dr. S.I. Stepanova and deposited at the Department of Nutriciology and Pharmaceutical Bromatology (National University of Pharmacy, Kharkiv, Ukraine). The herbal raw material was divided into three parts. The first part was dried at room temperature (18–22°C for 3–5 days), the second – at 60–70°C for 90 min, the third – at 50°C for 120 min (referred to as the ‘sample 1,’ ‘sample 2,’ ‘sample 3’ respectively). For the second and the third method, the original equipment presupposing mixed heat transfer was used [21,22].

2.2 Loss on Drying

3.0 g (accurately weighed) of the powdered raw material (mean particle size of approximately 10 mm) was placed in a weighing bottle previously dried under the conditions prescribed for the raw material to be examined, dried at 105ºC for 2 hours, cooled for 30 min in a desiccator and weighed. After this, drying and cooling were repeated (30 min for each process), until the difference between the two measurements did not exceed 10 mg. Loss of mass due to drying was expressed as per cent m/m [23].

2.3 HPLC Analysis

HPLC analysis was performed in accordance with [24], on an LC-20 Prominence Shimadzu

chromatograph equipped with a SPD-20AV detector, LC-20AD pump, SIL-20A autosampler, CTO-20A thermostat, CBM-20 ALITE systemic controller.

Herbal raw material was extracted with 40% ethanol (it has been shown that the maximal level of extraction of hydroxycinnamic acids is obtained just at this concentration [19]).

Phenolic compounds were separated using a 4.6 × 250 mm Spherisorb ODS-2 (5 µm) column.

The mobile phase composition was programmed as described in Table 1.

Table 1. The mobile phase composition in HPLC analysis

Time (min)	Mobile phase A, %	Mobile phase B, %
0–5	100	0
5–20	100→20	0→80
20–25	20	80
25–27	20→100	80→0
27–30	100	0

Mobile phase A content was as follows: 10% of acetonitrile and 90% of phosphate buffer solution (pH 2.5). Mobile phase B content was as follows: 90% of acetonitrile and 10% of phosphate buffer solution (pH 2.5). All solvents were of HPLC grade.

The column temperature equalled 40°C, mobile phase velocity – 1.0 ml/min, wavelengths 254 nm and 315 nm. Authentic standards of kaempferol 3-glycoside and chlorogenic acid were used.

2.4 Direct Spectrophotometry

The content of total hydroxycinnamic acid derivatives (expressed as chlorogenic acid) was determined using a direct spectrophotometry at 327 nm [19]. 0.5 g (accurately weighed) of the powdered raw material was placed in a 200 ml conical flask, 50 ml of 40% ethanol was added. The flask was weighed to 0.01 g, connected with the reflux condenser and heated in a water bath for 1 hour. After cooling the solution to room temperature, the flask was weighed, to restore the original mass 40% ethanol was added and mechanically stirred. The solution (solution A) was filtered through a paper filter, discarding the first parts of the filtrate. 5 ml of the solution A was diluted to 200 ml with 20% ethanol in a volumetric flask. Absorbance was measured on a spectrophotometer Specord 200 'AnalitykJena.'

2.5 Spectrophotometry of the Coloured Complexes of the Hydroxycinnamic Acids

To 0.5 ml of the solution A prepared as described previously, in a 10 ml volumetric flask 2 ml of 0.5 M hydrochloric acid was added as well as 2 ml of the solution prepared by dissolving 10 g of sodium nitrate and 10 g of sodium molybdate in 100 ml of water R. Then 2 ml of dilute sodium hydroxide solution R was added, diluted to 10 ml with water and mixed. The absorbance of the test solution was immediately measured at 525 nm, using as compensation liquid a solution prepared as follows: 0.5 ml of the solution A was mixed with 2 ml of 0.5 M hydrochloric acid and 2 ml of the dilute sodium hydroxide solution R, diluted to 10 ml with water R [23].

The content of total hydroxycinnamic acid derivatives, expressed as the chlorogenic acid was calculated from the following formula:

$$X = \frac{A \cdot K}{a \cdot E \cdot m},$$

A – Absorbance of the test solution;
a – volume of the test sample, ml;
K – Dilution factor;
E – Specific absorbance of chlorogenic acid (equals 531 – for 327 nm, 188 – for a coloured complex at 525 nm).
m – Mass of the raw material to be examined, g.

All the measurements were performed in triplicate.

3. RESULTS AND DISCUSSION

The use of plant raw materials in the manufacturing facilities is possible only if they are successfully preserved after harvesting, which is limited in time. The proposed method of goutweed preservation by adding salt [12] is unacceptable for pharmaceutical industry and for functional food products development. Additional intake of sodium, the excess of which in the diet of modern humans contributes to the development of 'diseases of civilization,' can not only eliminate the useful metabolic effects of GW, but also worsen the course of arterial hypertension, metabolic syndrome and other pathological states [25].

In this regard, a more acceptable alternative is the dehydration of raw materials (drying). The method of shade drying has been used for medicinal plants since time immemorial. Along with this, the equipment for artificial drying is being constantly developed. Thus, freeze-drying of the raw material provides certain advantages, including the better results for preserving phenolic compounds. This was shown, for example, for *Mentha spicata* L. leaves, being associated not only with the reduction in the loss of thermolabile compounds, but also with a facilitated extraction of phenolic compounds after the destruction of the cell walls by ice crystals [26]. Nevertheless, the equipment for freeze-drying is expensive, being not available for the most of farmers and suppliers. It has a relatively small productivity, making impossible its use for processing of substantial amounts of herbal raw materials in the places of vegetation.

Microwave- and infrared-assisted drying is highly effective, but it is characterized by uneven heating (due to the differences in absorption) with the destruction of biologically active substances, within the places of the local overheating. Besides, its energy demand is high.

A drying method with mixed heat supply (convective-conductive) has been proposed, which is characterized by the preservation of the biologically active substances in the final product (due to the low integral temperature effect on the raw materials, short term of drying, and the absence of direct contact with the stream of the drying agent) as well as optimal energy demand [21,22]. Just this method was chosen for our studies, proceeding from its economical availability and the suitability of the final product for many manufacturing processes, including production of galenic forms, as well as powders, which have favourable functional and technological properties and can be used in the composition of various food products (including functional foods). Along with this promising method, the method of shade drying, traditionally used for the most types of medicinal plant raw materials, was studied.

As can be seen from Table 2, the moisture content was comparable in all samples after their storage at room temperature (18–22°C), since a loss on drying did not differ between the samples. However, this content was reached significantly faster after artificial drying compared with shade drying (90 and 120 minutes and 3–5 days respectively).

To evaluate the phenolic compounds profile, HPLC method was used. Chlorogenic acid and the glycoside of kaempferol, probably trifolin, were detectable on the chromatograms of all of the samples (a typical chromatogram is shown in Fig. 1), while caffeic acid was practically not assessable. Thus, the profiles of the detected substances did not differ among the samples confirming the absence of the significant changes in the biologically active substances under all studied drying conditions.

However, the quantitative content of hydroxycinnamic acids, which are one of the main active components of goutweed, was markedly decreased after the use of the industrial drying methods – in the samples 2 and 3 (Table 2, typical spectra are shown in

Table 2. The influence of the drying conditions on the loss on drying and the content of total hydroxicinnamic acid derivatives in *Aegopodium podagraria* L. aerial part

		Loss on drying, %	Content of total hydroxycinnamic acid derivatives, expressed as chlorogenic acid, %	
			Spectrophotometry of the coloured complexes, 525 nm	Direct spectrophotometry at 327 nm
1	Sample 1 (drying at 18–22°C for 3–5 days)	7.80±0.33	4.03±0.13	3.74±0.10
2	Sample 2 (drying at 60–70°C for 90 min)	7.24±0.28	2.83±0.09	2.71±0.08
3	Sample 3 (drying at 50°C for 120 min)	7.59±0.35	2.06±0.07	2.27±0.07

Figs. 2 and 3).The lowest value was seen after drying at 50°C, while the temperature increases up to 60–70°C was more favourable for the preservation of hydroxycinnamic acids as well as for the decrease in the term of drying compared with the third method. Thus, industrial drying methods led to the obtaining of the samples with less hydroxycinnamic acids than shade drying. The question arises concerning the reasons of this phenomenon.

The influence of technological processes on the content of chlorogenic acid has been intensively studied in order to improve the methods of coffee beans (*Coffea arabica* L., *C. canephora* Pierre ex Fröhner) processing [27], sunflower seed (*Helianthus annuus* L.) protein isolates and related products production [28], as well as potato (*Solanum tuberosum* L.) tubers processing [29].

The data in the literature make it possible to exclude the degradation of hydroxycinnamic acids (mainly chlorogenic acid) into low molecular weight compounds, since these processes as well as lactone formation, epimerization and isomerization occur at temperatures above 200° C and are well studied in connection with the process of coffee roasting [27]. The absence of chlorogenic acid hydrolysis is also confirmed by the fact that caffeic acid was practically not assessable on chromatograms.

As noted in the works [28,30] within the temperature range used in our study during drying, two processes with the participation of caffeic acid esters (including chlorogenic acid) are principally possible: the non-enzymatic formation of the semiquinone-type product of oxidation (this reaction has been proven for the ester of caffeic acid in millimolar concentrations at pH 9.0–9.5, temperature 50°C, in the presence of oxygen [31] or through oxidation with polyphenol oxidase. The latter takes place at neutral pH values and is particularly probable under the conditions of our study. The structure and chemical properties of the products formed under the influence of polyphenol oxidase are studied in detail. Thus, dimerization occurs followed by condensation, in the presence of compounds with an amino group, a green dye is subsequently formed

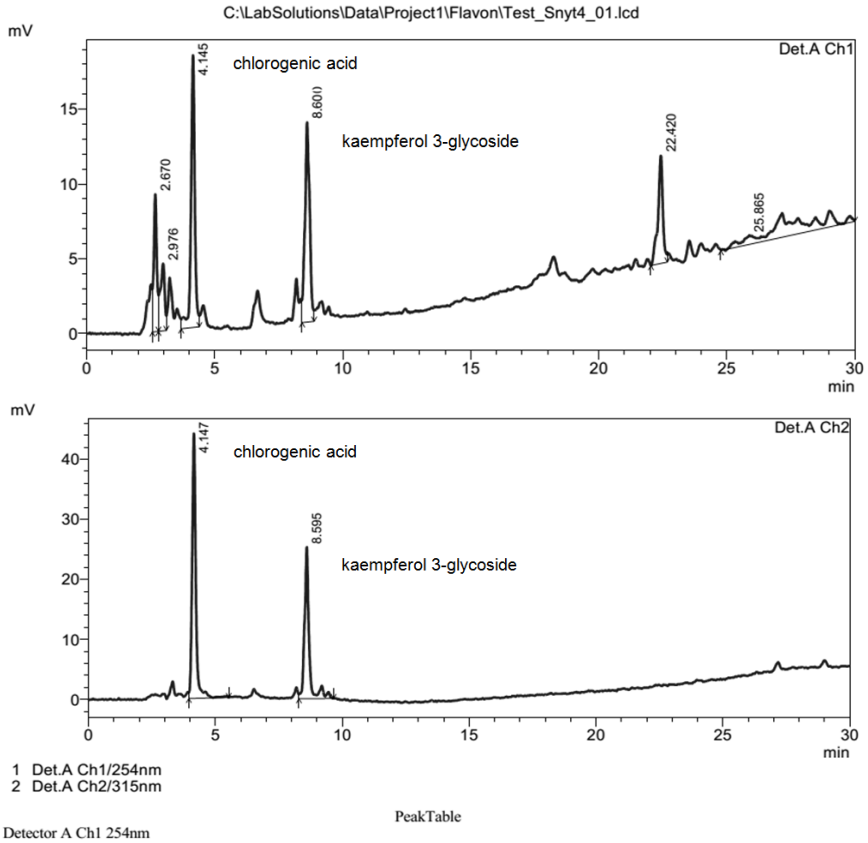

Fig. 1. Typical HPLC profiles of the *Aegopodium podagraria* L. aerial part extract
HPLC – high performance liquid chromatography

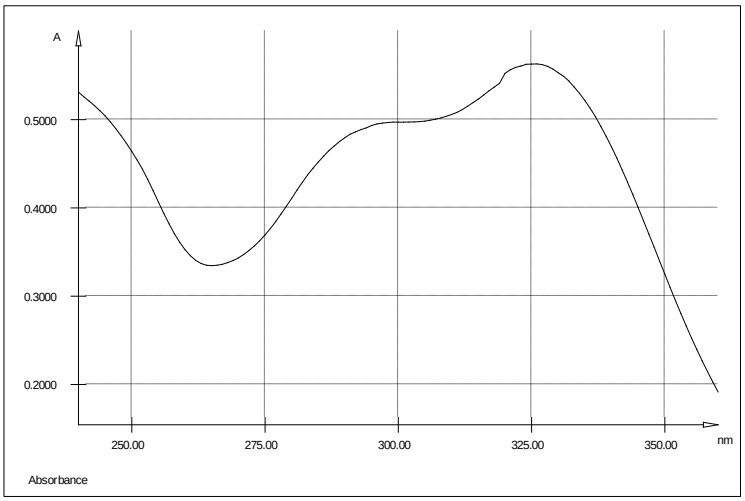

Fig. 2. Typical absorption spectrum of the *Aegopodium podagraria* L. aerial part extract (direct spectrophotometry)

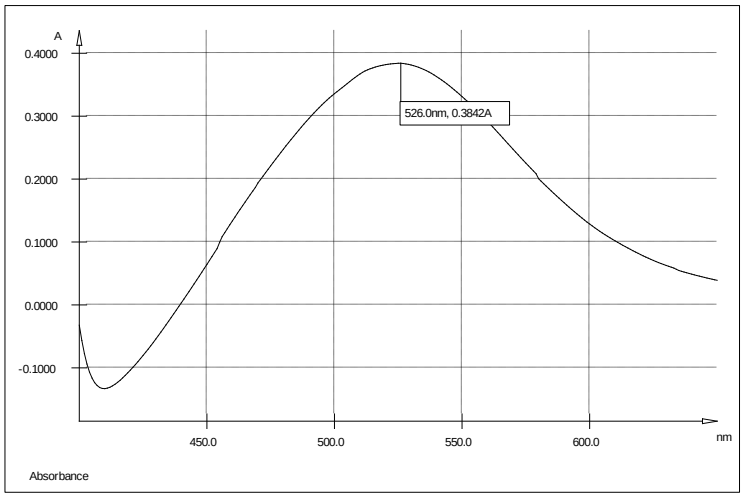

Fig. 3. Typical absorption spectrum of the *Aegopodium podagraria* L. aerial part extract with coloured complexes

(the process was intensively investigated in connection with the appearance of a characteristic colour in protein isolates of sunflower seeds) [31]. Of particular importance is the possibility of covalent bonds formation between these products and amino acids naturally occurring in plant tissues [30]. This reaction occurs intensively with glycine, histidine, and glutamine [32], which are present in goutweed raw material in significant amounts [14]. After the incubation of chlorogenic acid with polyphenol oxidase, a clear decrease in absorbance at 326 nm was observed [33], and after 3–5 minutes of this reaction the coloured product exhibiting absorption maximum at 420 nm was formed [34]. In with our results it should be noted that connection the optimum temperature for the functioning of polyphenol oxidase (although it is variable and depends on plant species and substrate) reaches 65 °C, while the activation of the enzyme, which is localized in an inactive form on the thylakoid membranes, occurs under the influence of damaging factors, dehydration and heating. Thermal inactivation of the enzyme during heating (within the temperature range 60–95°C) requires tens of minutes (depending on the plant species) and is accompanied with a primary increment of activity [35]. Therefore, it can be assumed that activation of polyphenol oxidase leads to a decrease in the content of chlorogenic acid during the initial period of drying, when the amount of water in plant tissues is still high. This is consistent with the fact that the content of

hydroxycinnamic acids is minimal in a sample dried at 50°C compared with the one dried at 60–70°C since dehydration and inactivation are likely to develop faster at higher temperatures.

The influence of these changes on the biological activity of the herbal raw material may be ambiguous. It is possible that dimer compounds and condensation products are extracted from the raw material, although not detected by the methods used in the study, and, if such extracts are ingested, their metabolism by the intestinal microflora may be expected. In the case of covalent bonds formation with amino acids, especially with the ε-amino groups of lysine and thiol groups of high molecular weight proteins [30], extraction can be problematic (similar to protein isolates of sunflower, of which some of these compounds are not extracted even by water [36], and the extraction by ethanol solution used in our study may also be inadequate). Furthermore, such compounds are not metabolized by humans [37,38]. On the other hand, the antioxidant activity of these substances was established in vitro [36] which may be of importance as a local effect in the gastrointestinal tract. Their ambiguous effect on the nutritional value of proteins is widely studied [38].

According to the data [15], ferulic acid is also present in goutweed raw material. Its participation in the synthesis of lignin and direct interaction with polysaccharides of the cell wall presupposes the changes of the content of its free form during the drying process. Besides, there are data in the literature that substantiate the possibility of changes in the quantitative ratio of the various polyphenols and secondary metabolites formation during the drying process (such phenomena were verified for the herbal raw material, for which hydroxycinnamic acids are one of the main active substances, namely the herb of *Echinacea purpurea* (L.) Moench [39]. Still we have not directly addressed these details in the present study.

The less significant contribution of polyphenol oxidase to the reduction in the content of hydroxycinnamic acids at the temperature 18–22°C (which could be assumed, proceeding from the much longer duration of the process compared with industrial drying and the possibility of enzymatic activity maintenance at such temperatures) is probably explained by the fact that the enzyme activation and release does not occur in the absence of abrupt rise of temperature and intensive dehydration, consequently its access to phenolic compounds of the cytosol and vacuoles is limited.

Thus, the oxidation of hydroxycinnamic acids (mainly chlorogenic acid) by polyphenol oxidase is probably the reason for the change in their content after the drying by industrial methods (at temperature 60–70°C and, especially, 50°C). There are data in the literature that partially support our results: the phenolic compounds profile of birch leaves was not changed principally after oven-drying (at 40 and 80°C), but there were no advantages of this method over shade drying concerning the concentration of phenolics [40], while for the plants of the family *Salicaceae* it was recommended to avoid oven-drying [41]. In the review [42] it is clearly stated that drying of raw materials containing phenolic compounds at 60°C should be avoided in connection with the maximum activity of polyphenol oxidase. This contrasts with the previously widespread approach to formulate general recommendations on drying regimes, when the temperature of 40–60°C was indicated as the optimal range for a wide spectrum of the herbal raw materials (with the exception of rose hips (*Rosa* L. spp.), digitalis (*Digitalis* L. spp.), and the species containing essential oils).

Thus, the research of the medicinal plant drying processes has intensified recently, leading to the development of the certain recommendations for each plant species and type of the herbal raw material. Our data form the first background for the development of the industrial drying methods for *Aegopodium podagraria* L. raw material, enabling its use for functional foods and drugs manufacturing (after the further studies, the directions of which are outlined below).

4. CONCLUSION

The obtained results allow to make the following conclusions:

1. The traditionally used shade drying of *Aegopodium podagraria* L. aerial part allows obtaining of the satisfactory quality of final product. This is consistent with previous results of the studies under the laboratory conditions. However, this method is unacceptable for use in the manufacturing facilities of the pharmaceutical and food industries, since it is not standardized due to the unavoidable differences in temperature

conditions and relative humidity of air, as well as microbial contamination and contamination with foreign particles.

2. The studied industrial drying methods do not cause significant changes in the spectrum of biologically active substances in *Aegopodium podagraria* L. aerial part, but they lead to a decrease in the quantitative content of hydroxycinnamic acids, which is presumably associated with the activation of polyphenol oxidase at the beginning of drying process.
3. Further search for the optimal drying conditions for the raw material of goutweed aerial part, in particular, it can be assumed that using a temperature above 90°C at the beginning of drying will contribute both to the inactivation of polyphenol oxidase and faster initial dehydration of raw material. Under such conditions, better preservation of hydroxycinnamic acids is expected. It is also expedient to study the changes in the quantitative ratio of polyphenols during the drying process, as well as to compare the composition of fresh and dried goutweed aerial part

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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