UDC 582.711.714:581.47:615.451.1:581.192:615.014.2
DOI: 10.15587/2519-4852.2023.274772

TECHNOLOGY OF OBTAINING AND CHEMICAL PROFILES OF DRY EXTRACTS FROM LEAVES OF PROSPECTIVE SPECIES OF THE HAWTHORN GENUS

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The aim of the work *was to develop a technology for obtaining dry extracts from leaves of non-pharmacopoeia species of hawthorn and establishing their chemical profiles.*
Materials and methods. *Leaves of Crataegus mollis Sarg., Crataegus submollis Sarg. and Crataegus arnoldiana Sarg., used to obtain the dry extracts, were harvested in the Botanical Garden of the National University named after M. N. Karazina in August 2021. To determine the chemical profiles of the obtained dry extracts, the spectrophotometric method and the high-performance liquid chromatography (HPLC) method were used.*
Results. *A technological scheme for obtaining dry extracts of hawthorn leaves has been developed. The content of flavonoids and hydroxycinnamic acids in dry extracts of the leaves of C. mollis Sarg., C. submollis Sarg. and C. arnoldiana Sarg. was determined. The content of free amino acids in extracts ranges from 0.70 % to 0.99 %. The content of flavonoids ranged from 7.25 %±0.04 to 8.43 %±0.01, of hydroxycinnamic acids – from 2.35 %±0.02 to 2.85 %±0.03. Rutin, kaempferol-3-O-glycoside, epicatechin and chlorogenic acid were identified in all extracts by the HPLC method. In dry leaf extracts of C. arnoldiana Sarg. and C. mollis identified p-coumaric acid; C. arnoldiana Sarg. – quercetin; C. arnoldiana Sarg. and C. submollis Sarg. – ferulic acid; C. submollis Sarg. and C. mollis Sarg. – quercetin-3-O-rhamnoside.*
Conclusions. *Dry leaf extracts of C. mollis Sarg., C. submollis Sarg. and C. arnoldiana Sarg. were obtained. For the first time, the content of flavonoids and hydroxycinnamic acids in the extracts was determined by HPLC. It was established that the chemical profile of the obtained extracts is formed by rutin, chlorogenic acid and kaempferol-3-O-glycoside, which can be used in further standardization of these substances. In addition, a comparative study of the amino acid composition of the obtained extracts was carried out for the first time*
Keywords: *hawthorn, leaves, dry extracts, chemical profile, technology, flavonoids, hydroxycinnamic acids, amino acids*

How to cite:
Sydora, N., Yakovenko, V., Ochkur, O., Rudnyk, A., Honcharov, O., Zuikina, S., Romas, K., Marchenko, M. (2023). Technology of obtaining and chemical profiles of dry extracts from leaves of prospective species of the hawthorn genus. ScienceRise: Pharmaceutical Science, 1 (41), 58–66. doi: http://doi.org/10.15587/2519-4852.2023.274772

1. Introduction

In the complex therapy of cardiovascular diseases, medicinal products of plant origin are used, which, along with other biologically active substances, contain hawthorn fruit liquid or dry extract [1, 2]. Hawthorn flower tincture is also used as an individual medicine.

The preparations based on hawthorn fruits have a cardiotonic, antihypertensive, and antiarrhythmic effect [3, 4]. In addition, Polyphenols, contained in herbal drugs, also exhibit antioxidant properties [5].

A sedative and coronaryolytic effect is more characteristic of the tincture of flowers, due to which this drug is used to relieve spasms of the coronary vessels of the heart, which is characteristic of angina pectoris and cardiovascular insufficiency [6].

The pharmaceutical market of Ukraine mainly offers liquid and tablet forms that contain an extract of the fruits of *Crataegus monogyna* L. Flowers with leaves of hawthorn (Flores cum folia *Crataegi* L.) and fruits (Fructus *Crataegi* L.) are included in the State Pharmacopoeia of Ukraine and the European Pharmacopoeia, however, it should be taken into account that the raw material base of *C. monogyna* L. in the territory of Ukraine is limited, since this species is endemic, which grows in the territory of Western Ukraine [7].

The national part of State Pharmacopoeia of Ukraine has included 12 hawthorn species that were from the SPh XI edition and did not have a raw material base in the territory of Ukraine. The natural areas of growth of these species are Central Asia, the Far East, and the Caucasus; that is, this raw material was relevant for the former USSR but is not an alternative for modern Ukraine [8].

However, it should be taken into account that in the wild and culture, the domestic flora includes more than 30 species of the hawthorn genus, which can be used as additional sources of biologically active substances (BAS).

Therefore, we believe the study of domestic, available species of the Hawthorn genus as sources of BAS is relevant.

As we reported earlier, when conducting chemo-taxonomic and morphological-taxonomic studies of leaves, fruits and flowers of non-pharmacopoeial representatives of the hawthorn genus, promising species for the further in-depth study were identified [9]. When studying the chemical composition of the leaves of representatives of the section *Molles* Sarg. belongs hawthorn genus, we have established that the raw material contains BAS of different chemical structures, including phenolic compounds, particularly flavonoids, which have a wide spectrum of pharmacological activity [10].

In order to obtain new plant substances and expand the use of domestic hawthorn species as sources of BAS, we consider it expedient to develop a technology for obtaining dry extracts of leaves of non-pharmacopoeial species and to establish their chemical profiles in order to further predict their pharmacological activity in view of the spectrum of BAS and with further implementation in production.

2. Planning (methodology) of the research.

The stages of obtaining dry extracts of hawthorn leaves and their research are shown in Fig. 1.

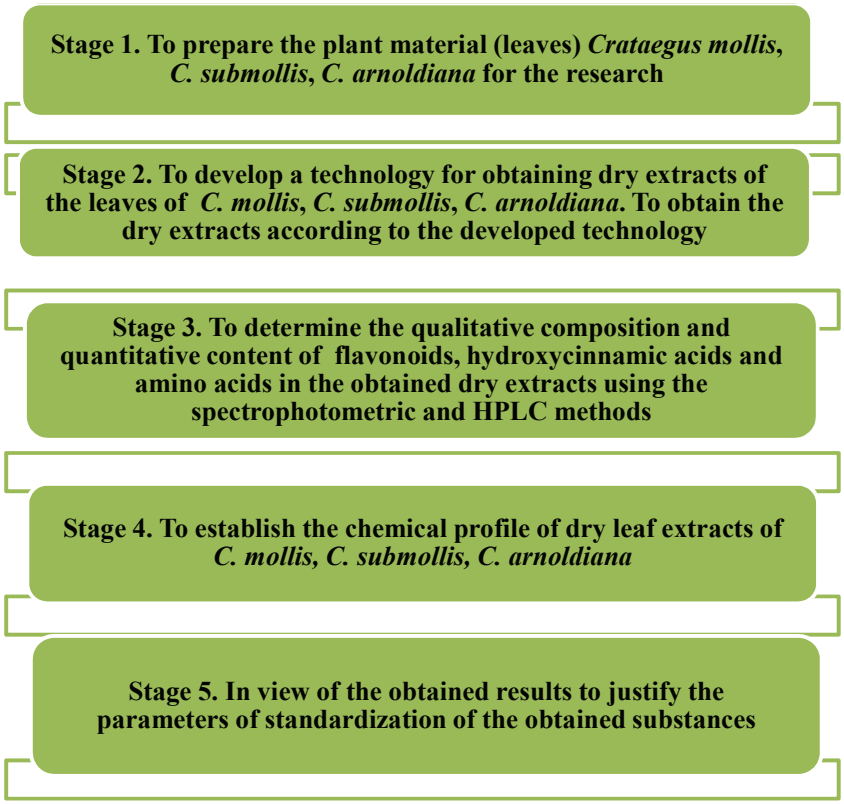

Fig. 1. The stages of obtaining dry extracts of hawthorn leaves and their research

3. Materials and methods

The subjects of the study were dry extracts obtained from the leaves of representatives of the section *Moles* Sarg. genus *Hawthorn* (*Crataegus* L.). Dry extracts were obtained from the leaves of *C. mollis* Sarg., *C. submollis* Sarg. and *C. arnoldiana* Sarg, harvested in August 2021.

The raw material base of these species on the territory of Ukraine is sufficient; they have been successfully cultivated as garden crops since 1860. Since the species of the Hawthorn genus are prone to hybridization, for the purpose of purity of the experiment and confidence in the belonging of the plants to a specific species, in order to prevent an error in their identification, we used samples of raw materials collected in the botanical garden of National University of V. N. Karazin (Kharkov). The identification of plants was carried out with the advisory help of the senior researcher of the botanical garden Shatrovskoy V. I.

In order to select the optimal extraction conditions and achieve the maximum yield of BAS, we determined the following technological parameters of raw materials: degree of grinding, the bulk density, the specific mass, the bulk volume, the fenestration, the porosity, the free volume of the layer (Table 1). The technological parameters of raw materials were determined according to the pharmacopoeial method [11].

In order to prevent the decomposition of BAS and the use of large volumes of 70 % *ethanol* P when developing the technology for obtaining extracts, we offer a percolation method that does not involve heating the raw materials during the extraction process.

Table 1

Technological parameters of hawthorn leaves

Parameters	Hawthorn species		
	C. submollis	*C. arnoldiana*	*C.mollis*
The bulk density, g/cm³	0.30± ±0.02	0.29± ±0.03	0.28± ±0.02
The specific mass, g/cm³	1.14± ±0.05	1.12± ±0.04	1.10± ±0.05
The bulk volume, g/cm³	0.34± ±0.03	0.36± ±0.02	0.32± ±0.01
The fenestration	0.11± ±0.02	0.19± ±0.02	0.12± ±0.03
The porosity	0.70± ±0.01	0.67± ±0.02	0.71± ±0.01
The free volume of the layer	0.73± ±0.05	0.74± ±0.02	0.75± ±0.02
The extraction absorption coefficient, ml/g			
Purified water	3.83± ±0.04	3.92± ±0.02	3.65± ±0.05
Ethanol 50 %	3.35± ±0.02	3.45± ±0.01	3.25± ±0.02
Ethanol 70 %	3.15± ±0.01	3.20± ±0.02	3.05± ±0.02
Ethanol 96 %	3.01± ±0.01	3.10± ±0.02	2.95± ±0.01

Obtaining dry extracts of hawthorn leaves.

The technological scheme for obtaining the dry extracts of hawthorn leaves is shown in Fig. 2.

The technological process of obtaining dry extracts consists of the following stages: grinding and sieving of plant raw materials; weighing raw materials; preparation of extractant; obtaining a liquid extract; evaporation of liquid extract; obtaining a dense extract; obtaining a dry extract; packaging and labelling; packaging of cans with extract in group containers.

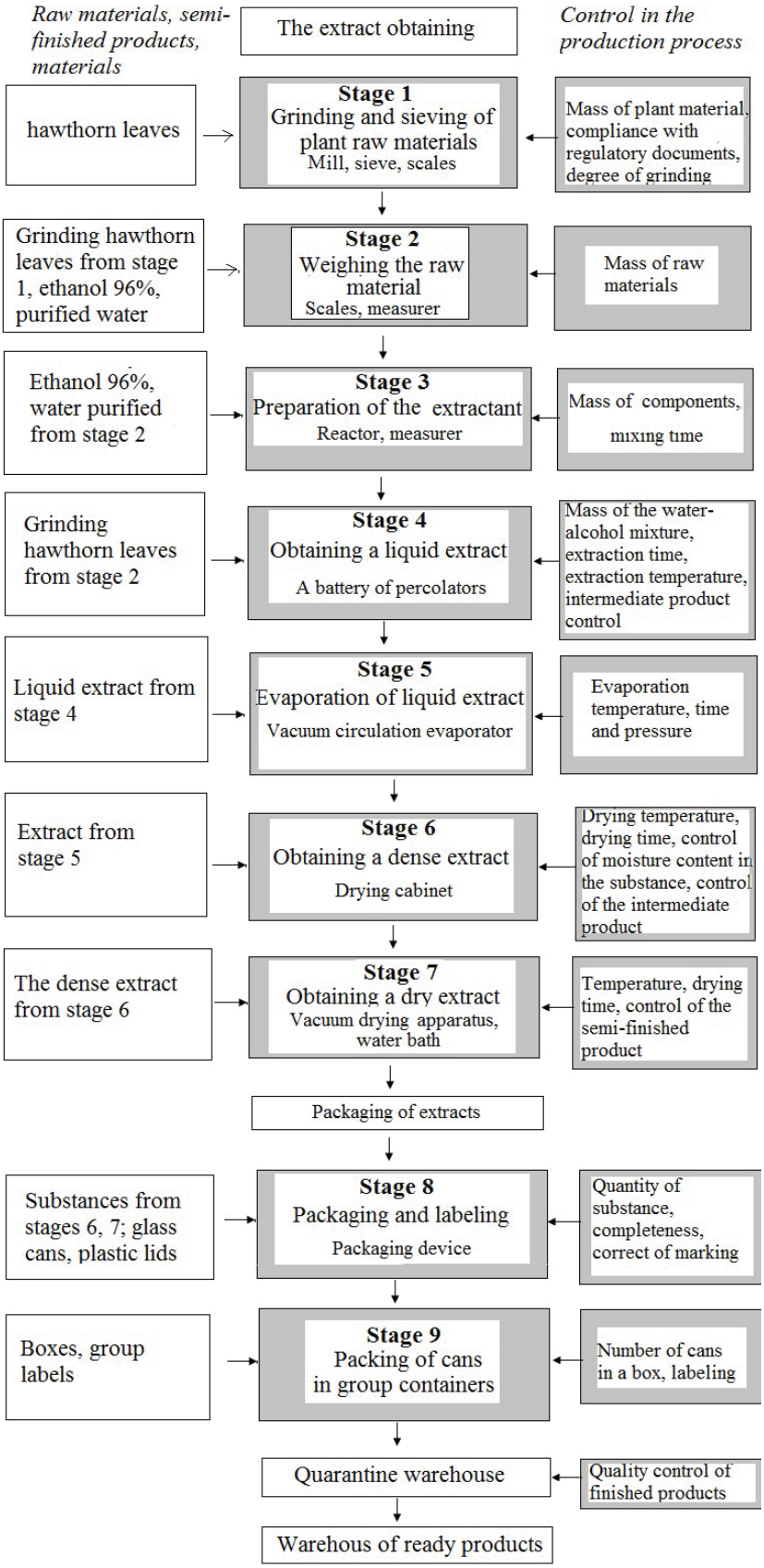

Fig. 2. The technological scheme for obtaining the dry extracts of hawthorn leaves

It should be noted that dry leaf extracts of *C. mollis*, *C. submollis* and *C. arnoldiana* were obtained separately in order to establish the chemical profiles of each substance.

Stage 1. *Grinding and sieving of plant raw materials.*

The leaves were ground on a PM-350 mills to a particle size of up to 3 mm, weighed and placed in fabric bags.

Stage 2. *Weighing the raw material.*

The hawthorn leaves, ethanol 96 % and purified water were weighed. Then, the raw materials were transferred to sealed containers and marked with labels.

Stage 3. *Preparation of the extractant.*

Ethanol 96 % and purified water from Stage 2 were transferred to the reactor, and the stirrer was turned on and mixed for 15 min. The obtained water-alcohol mixture was transferred to Stage 4.

Stage 4. *Obtaining a liquid extract.*

The liquid extract was obtained by the percolation method, and a battery of three percolators was used, into which equal parts of hawthorn leaves were loaded. 70 % ethanol was added to the first percolator until a "mirror" was formed and extracted for 24 hours (Extract 1). Extract 1 thus obtained was placed in a second percolator, and 70 % ethanol was added until a "mirror" was formed and extracted for 24 h. (Extract 2). The obtained Extract 2 was placed in the third percolator and extracted under the same conditions. A liquid extract was obtained in the ratio of raw material: extractant 1:1, which was poured into a collector and transferred to Stage 5.

The meal was unloaded from the extractor and sent for disposal.

Stage 5. *Evaporation of liquid extract.*

The liquid alcohol extract was evaporated on a vacuum circulation evaporator at a speed of 70.0 l/h. at a temperature of 65–70 °C and a pressure of 0.06–0.07 mPa up to 1/3 in relation to the original volume. The evaporated extract was poured into a graduated collector of cubic residue and transferred to Stage 6.

Stage 6. *Obtaining a dense extract.*

The extract from Stage 5 was dried in a vacuum oven at 70 °C for 45 min. up to 1 hour to the dense extract containing 15–25 % moisture [12].

Stage 7. *Obtaining a dry extract.*

Extracts from stage 5 were dried in a vacuum drying apparatus at a water bath temperature of 65–70 °C to a dry residue, which was then ground to a powdery state.

Stage 8. *Packaging and labelling.*

The obtained dry extracts were packed in sterile, tare-marked cans made of fibreglass with a neck and closed with plastic lids.

Stage 9. *Packaging.*

Marked cans with dry extracts were packed in group containers with group labels.

Study of the chemical profiles of the obtained extracts.

The extracts' hydroxycinnamic acids and flavonoids content was determined by the spectrophotometric method and the method of high-performance liquid chromatography (HPLC), amino acids – by the HPLC method [13, 14].

Spectrophotometric determination.

Hydroxycinnamic acids.

0.10 g of the extract was placed in a 10 ml pycnometer, was added 30 % *ethanol P*, was dissolved with shaking, adjusted to 10 ml with a solvent and stirred. 1 ml of the resulting solution was placed in a 10 ml pycnometer, and added 2 ml of a 0.5 *M solution of hydrochloric acid R*, 2 ml of a solution of 10 g of *sodium nitrite R* and 10 g of *sodium molybdate R* in 100 ml of *water P*, 2 ml of *sodium hydroxide* solution diluted *R*, *water R* was added to the mark [15].

Compensation solution. 1 ml of the stock solution was placed in a 10 ml pycnometer, mixed with 2 ml of 0,5 *M hydrochloric acid* R and 2 ml of *sodium hydroxide diluted solution R*, the volume of the solution was adjusted to the mark with *water R*, and stirred. Optical density was measured at a wavelength of 525 nm, as a comparison solution using a compensation solution.

The content of hydroxycinnamic acids *X* (%) in terms of acid chlorogenic is calculated by the formula:

$$X = \frac{A \cdot 10 \cdot 10}{m \cdot 188},$$

where *A* – optical density of the tested solution at a wavelength of 525 nm;

m – a mass of the test sample extract, in g;

188 – a specific indicator of chlorogenic acid.

Flavonoids.

About 0,25 g of extract was placed in a 25 ml volumetric flask, dissolved in 20 % *ethanol R* while stirring. The volume of the solution in the flask was brought to the mark with the same solvent and stirred (solution B). 2 ml of solution B added to a 25 ml volumetric flask and 2 ml of 3 % *aluminium chloride solution in* 96 % *ethanol R* was added, the volume of the solution was adjusted to 70 % *ethanol* R and stirred.

The optical density of the test solution was measured at a wavelength of 415 nm in a cuvette with a thickness of 10 mm.

Compared solution. A solution containing 2 ml of solution B is diluted to the mark in a volumetric flask of 25 ml with 70 % *ethanol R* and mixed.

Under the same conditions, conduct a test with 1 ml of a solution of rutin standard.

Compared solution. A solution containing 1 ml of a solution of the PhSS rutin is brought into a volumetric flask of 25 ml capacity with 70 % *ethanol R* to the label and mixed. Before measuring the optical density, the tested solution and the compared solution are filtered through a paper filter, and the first portions of the filtrate are discarded [16].

The content of the amount of flavonoids (%) was calculated by the formula:

$$X = \frac{A_1 \cdot a_0 \cdot 1 \cdot 25 \cdot 25 \cdot 100 \cdot 100}{A_0 \cdot a_1 \cdot 2 \cdot 25 \cdot 25 \cdot (100 - W)},$$

where *A* – optical density of the tested solution;

A_0 – optical density of the standard rutin solution;

a_1 – the mass of the test extract, in g;

a_0 – the mass of the standard solution of rutin, in g;

W – loss in mass during drying, in percentages.

Study of phenolic compounds by HPLC method.

The research was carried out on an Agilent 1200 3 DLC System Technologies chromatograph (USA), equipped with a Supelco DiscoveryC18 chromatographic column (250×4.6 mm), silica gel sorbent. Standards were introduced in a volume of 5 µl, tested extracts – 10 µl [17].

The chromatography was performed under the following conditions: mobile phase solution A – 0.1 % solution of H_3PO_4 in water, B – MeOH in ratios of 90:10, 70:30 or 20:80; the supply rate of the mobile phase is 0.25 ml/min.; working pressure of eluent – 240–300 kPa; the temperature of the column thermostat is 32 °C; sample volume – 5 µl; gradient mode (Table 2).

Detection parameters: measurement scale – 1.0; scanning time – 0.5 sec.; parameters of spectrum extraction – each peak is 190–600 nm [18].

Table 2

Gradient program

Time, min.	A% (0.2% TFA)	B% 70 % MeOH (0.2% TFA)	C% 100 %MeOH
0	92	8	0
8	62	38	0
24	0	100	0
24.1	0	0	100
29	0	0	100

Phenolic compounds were identified by the retention time of standards and spectral characteristics.

Identification of amino acids.

The determination method is based on the extraction of free amino acids from raw materials and acid hydrolysis with analysis of hydrolysates by HPLC with pre-column derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalaldehyde (OPA) followed by detection with a fluorescent detector.

Chromatographic separation of components was performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) under the following conditions: a Zorbax AAA column with a length of 150 mm and an internal diameter of 4.6 mm, a sorbent grain diameter of 3 µm; mobile phase A – 40 mM Na_2HPO_4 pH 7.8; B – ACN: MeOH: water (45:45:10, v/v/v); gradient separation mode: 0 min. – 12 % "B", 30 min. 25 % "B", 33 min. 25 % "B", 38 min. 30% "B", 40 min. 40 % "B", 41 min. 80 % "B", 49–60 min. 12 %; constant flow rate

1.5 ml/min.; the temperature of the column thermostat is 40 °C. Pre-column derivatization was performed automatically using FMOC reagent (Agilent 5061-3337) and OPA reagent (Agilent 5061-3335). The detection of amino acids was carried out using a fluorescent detector [19].

Sample preparation of raw materials. To determine free amino acids, a weight of powdered raw material was placed in a vial, and 2 ml of an aqueous solution of 1 *M hydrochloric acid* P was added; the mixture was kept in an ultrasonic bath at 50 °C for 3 hours.

Determination of total amino acids was carried out as follows: weight of raw material was placed in a vial, and 2 ml of an aqueous solution of 6 *M hydrochloric acid* P was added; the mixture was kept for 24 hours in a thermostat at a temperature of 110 °C (conducting hydrolysis). 0.5 ml of the hydrolyzate (pre-centrifuged) was evaporated on a rotary evaporator and washed three times with purified water P to remove hydrochloric acid. It was resuspended in 0.5 ml of purified water P and filtered through membrane cellulose filters with pores with a diameter of 0.2 μm. Obtaining fluorescent derivatives was carried out in automatic programmed mode before introducing the sample into the chromatographic column.

Amino acids were identified by comparing the retention times of compounds with a mixture of amino acid standards (Agilent 5061-3334). The quantitative content of amino acids was determined by the area of their chromatographic peaks. The content of bound amino acids was determined by subtracting the content of free amino acids from the total amount.

4. Results

Experimentally, it was established that the optimal degree of leaf grinding, which achieves the highest yield of extractive substances, is the particle size of 1.5–3 mm. 70 % (v/v) *ethanol* P was chosen as an extractant since it is when using this solvent that the maximum yield of phenolic compounds, in particular flavonoids, is achieved.

The obtained dry extracts are loose powders of yellow-brown colour with a weak specific smell. The results of the determination of the indicator «Loss in mass during drying» and the content of flavonoids and hydroxycinnamic acids in dry extracts by the spectrophotometric method are shown in Table 3.

It was established that the amount of flavonoids in the obtained extracts is from 7.25 % to 8.43 %, and hydroxycinnamic acids – are from 2.35 % to 3.45 %.

Table 3

Indicators of dry extracts of hawthorn leaves

Substance	Loss in mass during drying, % (*n*=3)	Content, % (*n*=5)	
		The sum of hydroxycinnamic acids	The sum of flavonoids
Leaves of C. *arnoldiana* dry extract	4.12±0.04	2.35±0.02	7.25±0.03
Leaves of C. *submollis* dry extract	3.15±0.03	2.85±0.01	8.43±0.01
Leaves of C. *mollis* dry extract	3.75±0.02	3.45±0.02	7.43±0.04

The highest amount of flavonoids was determined in the extract of the leaves of *C. submollis*, and hydroxycinnamic acids – in the extract of *C. mollis*.

Determination of the content of phenolic compounds by HPLC.

In the dry extract of the leaves of *C. arnoldiana* Sarg. the presence of 12 phenolic compounds was established (Fig. 3). The content of identified compounds was 4.1 %, of which flavonoids – 2.7 % and hydroxycinnamic acids – 1.4 %.

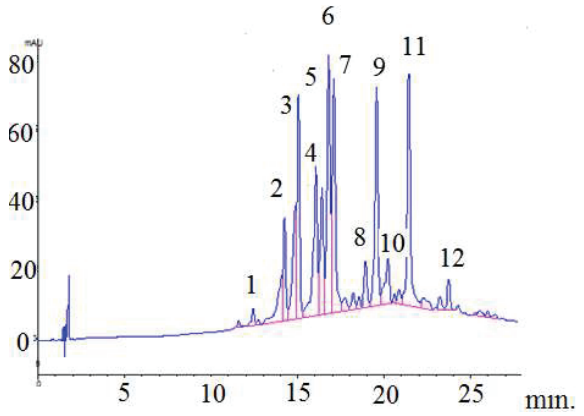

Fig. 3. HPLC chromatogram of phenolic compounds of dry extract of *C. arnoldiana* leaves

The content of phenolic compounds in the dry extract of *C. submollis* leaves is 4.45 %, of which 2.10% belongs to hydroxycinnamic acids, and 2.35 % to flavonoids. The presence of 10 compounds was established (Fig. 4).

In the dry extract of *C. mollis* leaves 8 phenolic compounds were determined (Fig. 5). The content of phenolic compounds was 4.1%, of which 1.3 % were hydroxycinnamic acids, and 2.8 % were flavonoids.

The compounds identified in the dry extracts of the leaves *of C. arnoldiana, C. submollis* and *C. mollis* are listed in Table 4.

Table 4

The content of phenolic compounds in dry extracts of the leaves of *C. arnoldiana, C. submollis* and *C. mollis*

Compound	Content, mg/100 g		
	Dry extract of leaf C. *arnoldiana*	Dry extrac of leaf C. *submollis*	Dry extract of *leaf C. mollis*
(-)-Epicatechin	155.40	125.80	125.30
Chlorogenic acid	1039.20	1635.20	1254.60
n- Coumaric acid	335.40	–	115.70
Ferulic acid	95.30	525.70	–
Rutin	1582.70	1125.30	1950.30
Quercetin	150.00	–	–
Kaempferol-3-O-glucoside	820.30	320.30	430.30
Quercetin -3-O-rhamnoside	–	725.10	225.60
The total amount of phenolic compounds	4178.3	4457.3	4101.8

Note: «–» – the compound was not detected

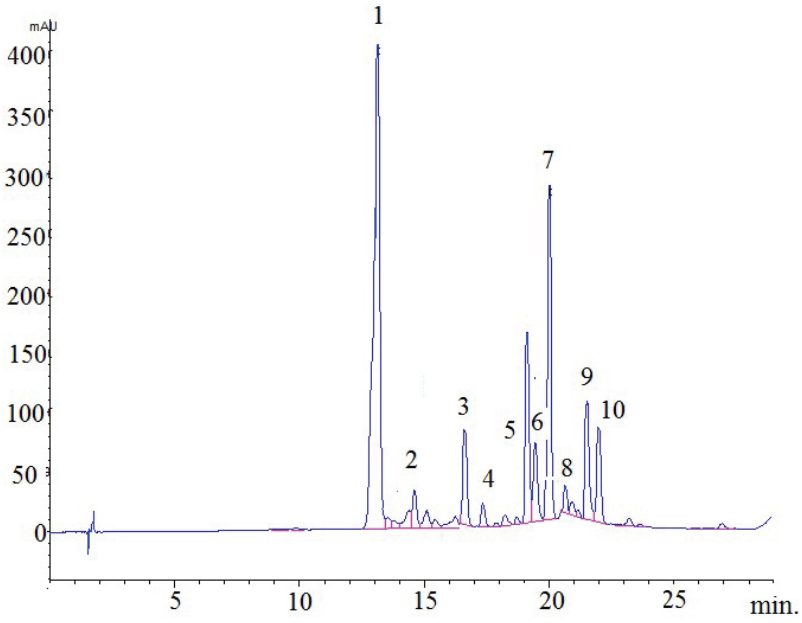

Fig. 4. HPLC chromatogram of phenolic compounds of dry extract of leaves of *C. submollis*

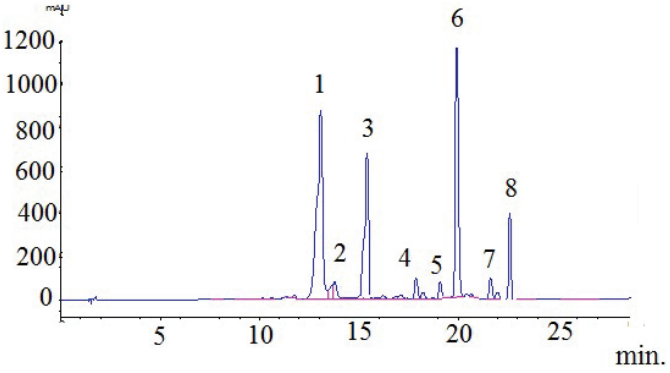

Fig. 5. HPLC chromatogram of phenolic compounds of dry extract of *C. mollis leaves*

As can be seen from the obtained results, flavonoid glycosides and chlorogenic acid prevail in terms of quantitative content in all extracts.

Rutin, chlorogenic acid, epicatechin and kaempferol-3-O-glucoside were identified in all tested extracts. The highest content of rutin from the total amount of identified phenolic compounds (in %) was found in the dry extract of the leaves of *C. mollis* (47.8 %); chlorogenic acid – in the dry extract of *C. submollis* leaves (36.6 %); kaempferol-3-O-glucoside – dry extract of *C. arnoldiana* leaves (19.6 %). The content of epicatechin in % of the total amount of phenolic compounds was from 3.05 % to 3.70 %.

The quercetin was identified only in the extract of *C. arnoldiana* leaves, which accounted for 3.5 % of the total compounds.

The n-coumaric acid was identified in the extracts of *C. arnoldiana* and *C. mollis*, whose mass fraction of the total content of phenolic compounds was 8.02 % and 2.8 %, respectively.

The quercetin-3-O-rhamnoside was identified in dry extracts of *C. submollis*, and *C. mollis* leaves, the content of which was 16.3 % and 5.5 % of the total amount of phenolic compounds, respectively.

Determination of amino acid content by HPLC.

16 amino acids were identified in dry leaf extracts: the content of free amino acids in *C. arnoldiana* leaf extract is 0.70 %, *C. submollis* – 0.80 %, *C. mollis* – 0.99 %. The highest content of free amino acids was determined for the dry extract of *C. mollis* leaves. All extracts contain high concentrations of glutamic acid and arginine, the content of which in terms of the total amount of amino acids (%) was: glutamic acid - from 17.8 % to 35.7 %, arginine – from 9.0 % to 43.6 %.

Serine, lysine and valine were identified in *C. arnoldiana* and *C. submollis* leaf extracts. Their content from the total amount of free amino acids was *C. arnoldiana* – serine (1.5 %), lysine (8.9 %), valine (1.4 %); *C. submollis* – 1.2 %, 5.3 %, 0.2 %, respectively. Chromatograms of free amino acids identified in the examined dry extracts are shown in Fig. 6–8.

The identified free amino acids are listed in Table 5.

As a result of the study of the amino acid composition of dry extracts of hawthorn leaves, essential (threonine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine) and replaceable amino acids (asparagine acid, serine, glutamic acid, proline, alanine, valine, tyrosine) were identified, which according to their chemical structure, they belong to sulfur-containing (methionine), diamino acids (lysine, arginine), cyclic (tyrosine), neutral (alanine, isoleucine, leucine, phenylalanine), amides of dicarboxylic amino acids (serine, threonine), monoaminodicarboxylic acids (glutamic and aspartic acids) and derivatives of glutamate (proline).

Table 5

Free amino acid composition of dry extracts of hawthorn leaves

Amino acid	Content, mg/100 g		
	Dry extract of leaf *C. arnoldiana*	Dry extract of leaf *C. mollis*	Dry extract of leaf *C. submollis*
L-Asparagine	74.2	135.8	96.3
L-Threonine	–	2.8	2.5
L-Serine	10.3	–	9.5
L-Glutamic	125.2	198.4	250.8
L-Proline	142.3	175.0	155.5
L-Glutamyne	17.0	3.5	5.2
L-Alanine	7.2	3.0	–
L-Valine	10.0	–	1.5
L-Methionine	34.5	1.5	4.0
Isoleucine	48.0	0.9	1.95
L-Leucine	36.0	3.5	0.7
L-Tyrosine	10.0	14.0	7.0
L-Phenylalanine	9.5	13.5	6.5
L-Histidine	15.0	6.0	13.5
L-Lysine	63.0	–	42.15
L-Arginine	98.4	432.8	203.4
Total	700.6	990.7	800.95

Note: «–» – the compound was not detected

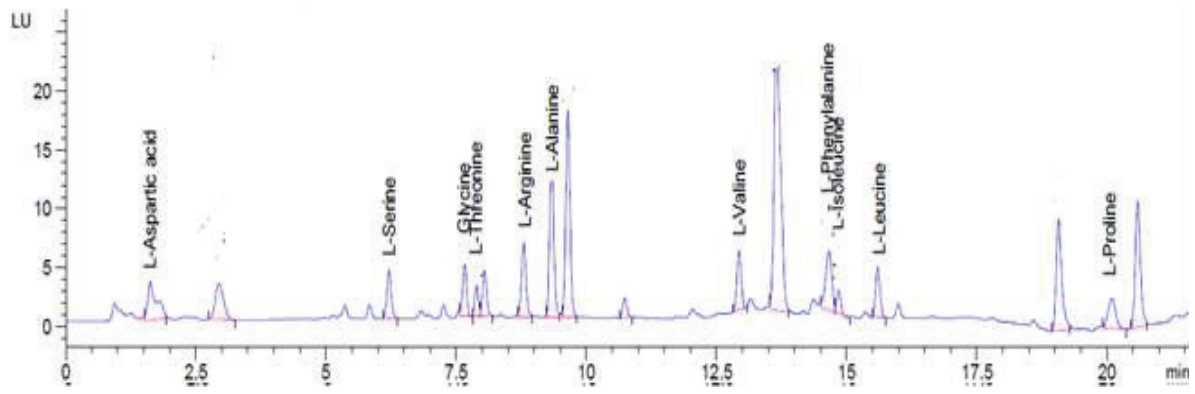

Fig. 6. The chromatogram of free amino acids of dry extract of *C. arnoldiana* leaves

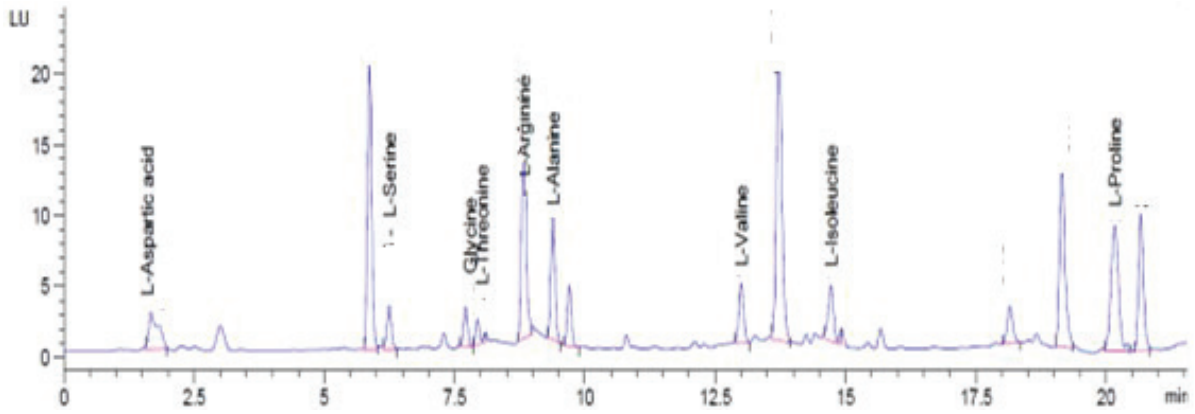

Fig. 7. The chromatogram of free amino acids of dry extract of *C. submollis* leaves

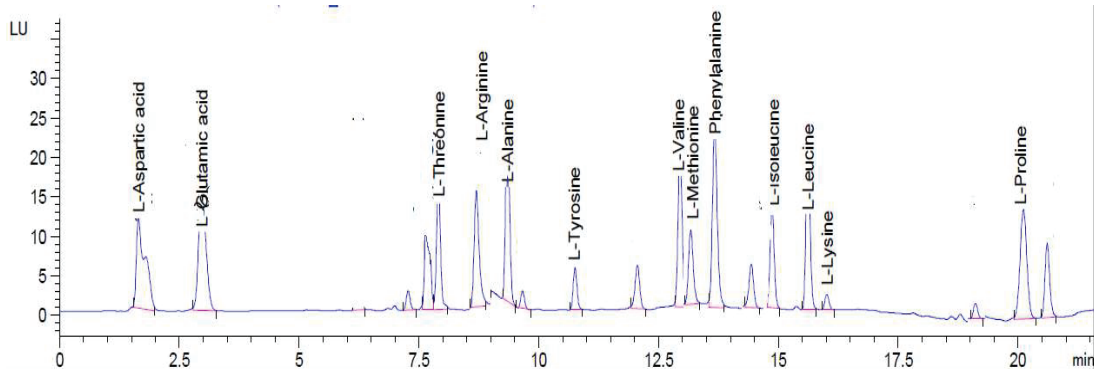

Fig. 8. The chromatogram of free amino acids of dry extract of *C. mollis* leaves

The obtained results can be used in the future for the standardization of the obtained substances and in the development of their quality control projects in accordance with the pharmacopoeial requirements.

5. Discussion of research results

The technology for obtaining dry extracts of hawthorn leaves, representatives of the section *Molles* Sarg, was developed for the first time and established parameters of their standardization.

The obtained dry extracts meet the requirements of PhEur 7 ed., 2010 (2.2.32) according to the «Loss in mass during drying» indicator, according which this indicator should not exceed 6 %.

The chemical profile of the extracts was established; common compounds are chlorogenic acid, epicatechin, rutin and kaempferol-3-O-glucoside.

The amount of flavonoids (%) in dry extracts is from 7 % to 8 %, which meets the requirements of EuPh (1432), which for dry extracts should be at least 6%.

Chlorogenic acid, identified in all extracts, exhibits antioxidant, hepatoprotective and hypotensive properties [20]. Rutin and quercetin have a capillary-strengthening effect [21]. In an experiment, kaempferol and its glycosides have a vasodilating effect and lower blood cholesterol [22].

Amino acids in combination with other BAS have different pharmacological effects: they affect the cardiovascular system, have hypoglycemic, anti-aggregate, hepatoprotective activity, normalize the exchange of vitamins and enzymes, and channelize oxidative processes. As can be seen from the obtained results, the extracts are characterized by the accumulation of amino acids that have a positive effect on the cardiovascular (glutamic and aspar-

agine acids, leucine, isoleucine, arginine, proline, lysine) and nervous systems (valine), which can be taken into account in the future development of medicines.

Research limitation. During the study of phenolic compounds by the method of high-performance liquid chromatography, not all compounds that were visualized on the chromatogram were identified. That is, we were limited in terms of the availability of standard samples.

Prospects for further research. Taking into account the chemical composition of the obtained dry extracts, we believe that in the future it is appropriate to study their pharmacological activity, in particular antihypertensive, which can be used in the production of medicines for the treatment of cardiovascular diseases, in particular, hypertension. We consider it appropriate to use these substances as part of medicines that can be used both for prevention and complex therapy of conditions accompanied by increased blood pressure and coronary spasm.

6. Conclusions

The main technological parameters of the leaves of *C. submollis*, *C. mollis* and *C. arnoldiana* have been established. The technology for obtaining dry extracts was developed and their chemical profile was established. The quantitative content of the sum of flavonoids in the extracts was determined by the spectrophotometric method, which in % was from 7.25±0.03 to 8.43±0.01 and hydroxycinnamic acids - from 2.35±0.02 to 3.45±0.02.

Epicatechin, chlorogenic, *n*-coumaric and ferulic acids, rutin, quercetin, kaempferol-3-O-glucoside, quercetin-3-O-rhamnoside were identified by HPLC in the extracts. Based on the total content of phenolic compounds, hydroxycinnamic acids are made up from 35 % to 48 %, flavonoid glycosides from 49 % to 64 %, and flavonoid aglycones from 3 % to 8 %. By using HPLC method, 16 amino acids were identified.

Due to the content of phenolic compounds and amino acids, the dry extracts of leaves of *C. submollis*, *C. mollis* and *C. arnoldiana* are promising substances for further research and for implementation to create a new medicines.

Conflict of interests

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 article.

Funding

The study was performed without financial support.

Data availability

Data will be made available on reasonable request.

Acknowledgements

We are grateful to the senior researcher of the Botanical Garden of the National University M. N. Karazin, Shatrovskoy V. I. for the botanical evaluation and identification of the studied plant and raw materials.

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Received date 08.12.2022
Accepted date 16.02.2023
Published date 28.02.2023

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