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Article *in* Biotechnologia Acta · December 2021

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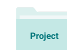

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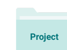

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SIMULTANEOUS IDENTIFICATION, QUANTIFICATION, AND MAIN COMPONENTS ANALYSIS OF *Artemisia annua* AND *Artemisia tilesii* "HAIRY" ROOT EXTRACTS

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Received 18.09.2020
Revised 28.11.2021
Accepted 30.12.2021

Aim. The profiles of polyphenolic phytochemicals in extracts of "hairy" roots of *Artemisia tilesii* Ledeb. and *Artemisia annua* L. were studied. Analytical separation and quantification of main components in extracts were evaluated.

Methods. "Hairy" roots were grown *in vitro* on Murashige and Skoog medium. High-performance chromatography coupled with different types of detection (photo diode array detection (DAD) and electrospray ionization with ultra-high resolution Qq-Time-of-Flight mass spectrometry) was used to identify and quantify the main biologically active components in ethanol extracts of "hairy" roots.

Results. The amount of flavonoids was 94.71–144.33 mg RE/g DW and 33.52–78.00 mg RE/g DW in "hairy" roots of *A. annua* and *A. tilesii*, respectively. In most samples of "hairy" roots, the amount of flavonoids was higher than the content in the control plant roots. The presence of Apigenin (0.168 ± 0.003 mg/L and 0.178 ± 0.006 mg/L), Quercetin (0.282 ± 0.005 mg/L and 0.174 ± 0.005 mg/L) in the extracts of *A. annua* and *A. tilesii* was shown by reverse-phase HPLC-DAD method. Chlorogenic acid, Kaempferol, and other flavonoids were detected.

Conclusions. Developed HPLC-DAD method demonstrated the high percentage of recovery, low limit of detection and quantification (9,11 ng/ml ≤ LOQ ≤ 16,51 ng/ml). Thus, the method is suitable for the simultaneous quantification of phenolic acids and flavonoids in various plant extracts with short time and high efficiency.

Key words: *Artemisia tilesii*, *Artemisia annua*, polyphenols, flavonoids, "hairy" roots, reversed-phase HPLC with diode matrix detector.

In recent years, polyphenolic compounds have aroused increasing interest in the food and medical industries due to their positive and "mild" effects on human health [1]. Phenolic compounds accumulated in crops, fruits, and vegetables are associated with a wide range of health benefits, such as antioxidant, antidiabetic, anti-inflammatory, and antitumor properties. That is why an extraction of bioactive compounds from plant raw materials is intensively studied now [2]. Increased interest in this area is due to the broad spectrum for medical applications or plant-derived chemicals. These compounds play an important role in maintaining the balance of redox processes in cells and prevent the damaging effects of free radicals. In particular, the flavonoid epicatechin is a powerful antioxidant [3]. Compounds such as rutin [4], quercetin [5], luteolin [6, 7], apigenin [8] have antioxidant properties, and also demonstrate anti-inflammatory effects. Thus, flavonoids synthesized in plants can be used in the treatment of inflammation of various origins.

Chemical analysis of ethanolic and aqueous extracts of various *Artemisia* genus plants showed the presence of active compounds

such as flavonoids, terpenes, proteins, polysaccharides, coumarins, and alkaloids [9]. For example, *A. annua* plants are known to produce artemisinin with antimalarial properties [10]. Our research demonstrated the accumulation of polyphenols in plants of *A. tilesii*, the study of which is of particular interest due to the peculiarities of their physiology. At the same time the chemical composition, quantitative analysis of ethanolic extracts of *A. tilesii* plants, and also the effect of *Agrobacterium*-mediated transformation on the synthesis of different metabolites in the transgenic roots of these plants are insufficiently studied.

Genetic transformation using *Agrobacterium rhizogenes* is a well-known method used to obtain "hairy" root culture. In the process of transformation, bacterial *rol* ABCD genes are transferred to plant cells. These bacterial genes are known as inducers of changes in secondary metabolism [11, 12]. Due to this feature of *rol* genes, the process of genetic transformation can be used as a way to obtain superproducer lines for "green" synthesis of valuable biologically active compounds. The possibility of long-term cultivation of "hairy" roots, characterized by the ability to grow in the medium without the addition of specific regulators, opens the way to mass production of such roots in bioreactors and thus obtaining valuable raw plant materials. Cultivation in bioreactors allows to produce these roots throughout the year, to eliminate the dependence of the synthesis of bioactive compounds on the vagaries of weather and environment (such biosynthesis in nature depends on temperature, humidity, soil pH, lighting, etc.), reduce the cost of raw materials because high-cost reagents, lighting, and additional heating are not required in the process of cultivation.

The increase in the total content of polyphenols in plant tissues due to the peculiarities of the influence of the named above bacterial *rol* genes on secondary metabolism attracts considerable attention of researchers. Though, it is important to optimize the method of efficient extraction (solvent type, temperature of extraction process, etc.) and quantify the composition of extracts from plant material of different origins in order to qualitatively and quantitatively analyze them. To quantify polyphenols and phenolic acids in plant extracts, a significant number of high-performance liquid chromatographic (HPLC) techniques are proposed, which include UV and MS detection (HPLC-MS) [13, 14]. However, the high cost of HPLC-MS makes HPLC techniques with a UV or diode array detection (HPLC-DAD) more convenient [15–17]. Though, the techniques used to analyze plant extracts by HPLC have several disadvantages, such as long run times, low resolution, and low efficiency.

The study of polyphenols content and development of a method of effective extraction and quantification of bioactive compounds in the *A. tilesii* and *A. annua* "hairy" roots were the aim of the work. Ultra high-performance chromatography coupled with electrospray ionization with ultra-high resolution Qq-Time-of-Flight mass spectrometry (UPLC-ESI-UHR-Qq-TOF-MS method) was used for the identification of biologically active compounds, including flavonoids and other polyphenols. Simultaneous quantification of polyphenols in the ethanol extracts of "hairy" roots was investigated by reversed phase HPLC-DAD.

Materials and Methods

Reagents. Standard references (>98%) were used, including chlorogenic acid (00500590-25mg, primary reference standard), apigenin (10798-25 mg, ≥ 97.0% (HPLC)), rutin (78095-25 mg, analytical standard), quercetin (PHR1488-1g, pharmaceutical secondary Standard; Certified Reference Material), luteolin (72511-10 mg, analytical standard), kaempferol (60010-25 mg, ≥ 97.0% (HPLC)), kaempferol-3-glucoside (6843-5 mg, analytical standard)) obtained from Sigma-Aldrich. All standards were prepared in 2% formic acid in MeOH (1 mg/mL). Working solutions were prepared by diluting standard solutions to a concentration of 0.01–0.05 mg/mL. The solvents (CH_3OH, CH_2Cl_2, EtAc, CH_3CN) used for the chromatographic analysis were HPLC-grade (Sigma-Aldrich, Spain). Ultrapure water was provided by a Milli-Q® purification system (Millipore, USA).

Plant material. The "hairy" roots of wormwood (*Artemisia tilesii* and *A. annua*) from the collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine were used. The roots were cultivated *in vitro* on solidified Murashige and Skoog nutrient medium with halved macrosalt content. The cultivation time was three weeks at temperature of +24 °C. The roots were separated from the medium, washed with distilled water, lyophilized and powdered.

Preparation of extracts. To prepare the extracts, previously dried and powdered

"hairy" roots were extracted with 70 vol. % ethanol (100 mg (EtOH)/20 ml (H_2O)) during two days on a rotary shaker at +28 °C.

Total flavonoids content assay. The standard spectrophotometric method [18] was employed using the Fluorate-02-Panorama (Russia). 0.25 ml of the sample of each extract was mixed with 1 ml of double-distilled water and 0.075 ml of 5% $NaNO_2$ solution, and allowed to react for 5 min at room temperature. After that, 0.075 ml of 10% $AlCl_3$ solution was added. After another 5 min of incubation, 0.5 ml of 1 M NaOH solution and 0.6 ml of double-distilled water were added to the reaction mixture. The absorbance of the sample was measured at 510 nm. The total flavonoid content was expressed as milligrams per gram of dry root weight in rutin equivalent (mg RE/g DW). The amount of flavonoids in extracts was performed by calibration curves in the concentration range from 50 µg/ml to 500 µg/ml: $y = 0,8842x — 0,0606$ ($R^2 = 0,9988$).

Sample preparation of extracts for chromatographic studies. Ethanol extracts from "hairy" roots were centrifuged at 5000 rpm for 10 min to precipitate a solid fraction (if it was presented). Acid hydrolysis was performed by adding an equal amount of 1M HCl to the methanolic extract (1 ml: 1 ml) for 1 hour without heating. The extracts were diluted with 0.5 ml of 1% (v/v) solution of formic acid in MeOH, and filtered using a membrane filter (nylon) (diameter: 13 mm, pore size: 0.45 mm) and stored at 6 °C until HPLC analysis.

Identification of biologically active compounds in the extracts. The UPLC system (Dionex Ultimate 3000) with electrospray ionization and ultra-high resolution Qq-Time-of-Flight mass spectrometry (Bruker Impact II) was used for chemical compositions analysis of the ethanol extract of "hairy" roots. The mass spectrometer was operated in the negative ESI mode with Duo-Spray source, and the mass scan range was set at m/z 50–2 500 for both TOF-MS and TOF-MS/MS scan with 2 700 resolution. The following parameter conditions were used: ion spray voltage, 3500 V; ion source heater, 500 °C; curtain gas, 25 psi; collision energy, 10 eV; declustering potential, 100. The identification of polyphenolic compounds in extracts was determined based on their mass fragmentation pattern, low mass error within the acceptance range of ± 5 mDa, and ion response. The analyst TF software (version 1.7) combined with the information-dependent acquisition packing was used to acquire the MS/MS data.

The mobile phase was composed of 0.1% formic acid in water (elution A) and methanol (elution B) using a gradient elution: 30% B (0–5 min), from 30% to 50% B (5–20 min), from 50% to 90% B (20–40 min), and from 90% to 100% B (40–45 min).

Procedure of quantitative determination of polyphenolic compounds in the extracts. Chromatograms of the extracts were recorded using a HPLC-DAD method (Shimadzu LC-20). Optimized chromatographic conditions for the quantification of flavonoids and phenolic acids in the gradient mode were used with a solvent system: mobile phase A: 1% (v/v) formic acid in methanol/water (25/75, v/v); mobile phase B: acetonitrile. Gradient: from 0 to 30 min, 100–64% A; from 30 to 31.2 min, 64–0% A; from 31.2 to 40.2 min, 0% A; from 40.2 to 42.5 min, 0–100% A; from 42.5 to 47.4 min, 100% A. The flow rate was 0.5–1.0 ml/min and the injection volume was 12 µl. The absorption spectra were recorded at 255 nm. Column Zorbax Eclipse Plus® C18 (15 cm, 3.0 mm, 3 µm) was used with Phenomenex pre-column (Gemini® NX C18 (4×3.0 mm)). The analysis was performed with column temperature at 28 °C. The total analysis time of the sample was 65 min.

Validation of the method. According to the recommendations of ICH/2005/Q2/R1 [19], there are various parameters for testing the reproducibility of the method, namely: efficiency, limit of detection (LOD), limit of quantification (LOQ), linearity, accuracy, and precision. Calibration curves were constructed based on the average peak areas of the five concentrations (0.1, 0.05, 0.01, 0.005, and 0.001 mg/l) of each of the six standard solutions (in three injections) measured in three parallels at wavelength 255 nm. We used concentration ranges 0.025–100 mg/l and 0.05–200.0 mg/l.

To evaluate the accuracy of the method for the determination of polyphenolic compounds recovery-test was used. Two different amounts of polyphenolic compounds (1.5 and 0.15 mg, a solution of 1% formic acid in methanol) were added to extracts before extraction. The analyte content was evaluated in three volumes for each added amount. Preliminarily, the absence of detectable components in the extracts was evaluated. Recovery (%) values were calculated using formula:

$$R = [(C_{found} - C_{contained}) / C_{added}] \times 100.$$

The high recovery-test was observed at 96–103%.

The slopes, intercepts, and the determination coefficients of each polyphenols

were calculated using last square linear regression analysis. LOD and LOQ values for each of studied compounds were evaluated according to ICH guideline [19] and were calculated using the following formulas:

$$LOD = 3\sigma/b;$$
$$LOQ = 10\sigma/b$$

where σ is the standard error of the intercept α; and b — slope of the calibration curve.

Statistical analysis. The analytical data were shown as means of triplicates and subjected to variance analysis using R software (version 4.0.4). The results were expressed as mean ± standard deviation at $P = 0.95$. Linear regression method was applied and coefficient of determination (R^2) was calculated for establishing the relationship between the values.

Results and Discussion

Estimation of the chemical composition of "hairy" root extracts.

Four lines of "hairy" roots of *A. annua* and six lines of *A. tilesii* were used in the study. The "hairy" root samples of *Artemisia tilesii* and *Artemisia annua* are presented in Fig. 1.

Various methods are used now for the extraction of bioactive compounds, including flavonoids, from plant raw materials to obtain the maximum possible concentrations of the target components by liquid extraction. Several solvents are commonly used to extract these phytochemicals. Ethanol and methanol are the most common ones to extract flavonoids, while water is often used to remove polar compounds from plant material. Ethanol is preferred in most studies, probably because of its safety, availability, and efficacy [20].

Due to the fact that there may be significant differences in the content of polyphenolic compounds in different lines of "hairy" roots owing to the indeterminate site of introduction of *rol* genes, it is necessary to conduct screening studies of the chemical composition of extracts including total flavonoids content to select the most productive samples (Fig. 2).

The primary analysis of the total flavonoid content in extracts from "hairy" root lines revealed significant variability in this parameter. Thus, the content of flavonoids in the roots of *A. annua* and *A. tilesii* varied within 94.71 ± 14.7–144.33 ± 28.1 mg/g dry weight (DW) and 33.52 ± 1.9–78.00 ± 4.9 mg/g DW, respectively. The content of these compounds in the "hairy" roots of *A. annua* was 2.17–3.32 fold higher than the content in the roots of the control plants grown in the same conditions *in vitro* (Fig. 2, samples Aa 1–4).

In three of the studied samples of *A. tilesii*, the total content of flavonoids differed little from the control parameter (Fig. 2, samples At 1–3). However, in other lines of "hairy" roots, the concentration of flavonoids was 1.78–2.43 fold higher than in the control sample (Fig. 2, samples At 4–6). Thus, transformation using *Agrobacterium rhizogenes* has indeed led to a significant stimulation of flavonoid synthesis in some "hairy" root lines. Since wormwood is a medicinal plant, it was of the special interest to study the obtained extracts and determine the qualitative and quantitative content of polyphenolic compounds extracted from this plant material.

Identification of biologically active compounds in ethanolic extracts of "hairy" roots. Among the available modern chromatographic methods, high-performance chromatography in combination with a hybrid pulsed tandem quadrupole time-of-flight mass spectrometer was recognized as a promising analytical method for chemical

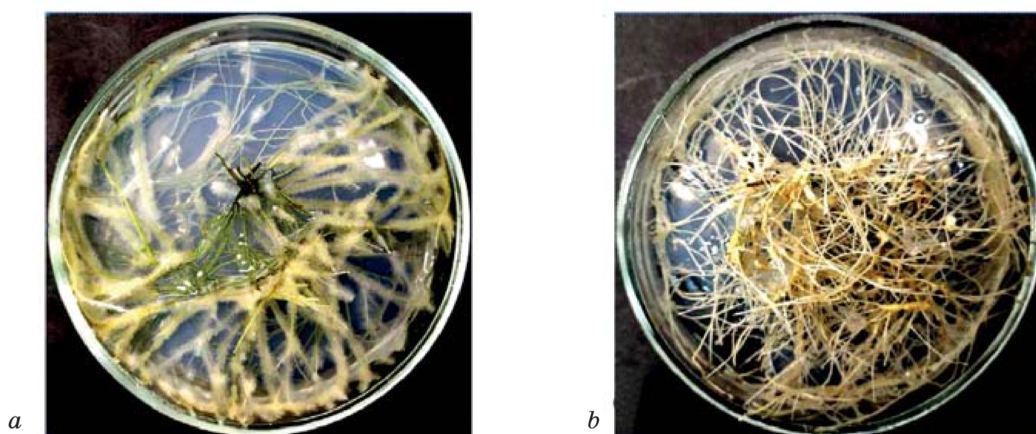

Fig. 1. **The "hairy" roots of *Artemisia tilesii* (*a*) and *Artemisia annua* (*b*) grown *in vitro***

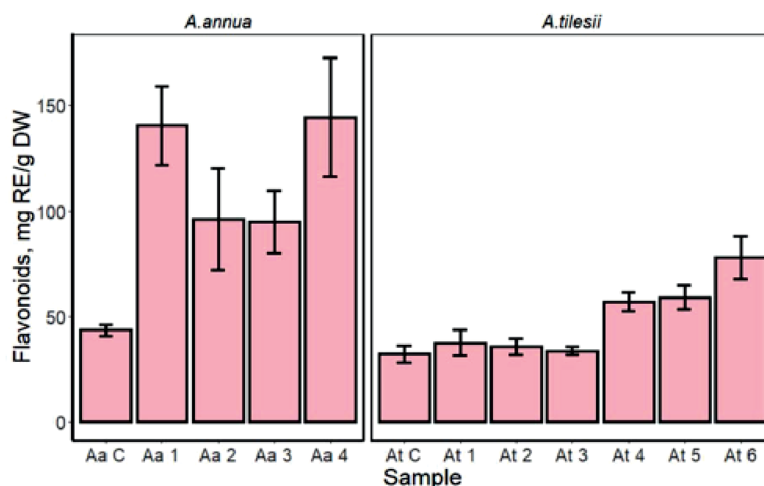

Fig. 2. **Total flavonoids content in ethanol extracts of different lines of *Artemisia annua* and *A. tilesii* "hairy" roots**

screening and identification of major chemical components of plants [21]. In addition, during one analysis, the detection of positive and negative ions is possible by switching the polarity of the ionizer, conductive system and Qq-TOF analyzer. Using this method, it is possible to detect simultaneously and identify bioactive compounds in the extracts according to accurate measurements of the mass of fragments of the components of the extract, as well as the system of library analysis of the obtained mass spectrometric data. Electron spray ionization and Qq-TOF, which allows measurements in TOF mode and MS mode, were used to detect and identify bioactive compounds. Measurements using TOF mode were performed with high resolution and accurate mass determination, as well as with a high speed of mass array analysis in their entire spectrum. In this mode, the detector registered the time and number of ions as they leave the flight tube, which improves the separation of the components that are part of the extract and need different times to reach the detector. Using the MS-MS mode with quadrupole and hexapole (hexanol high-frequency collision cell), mass spectra measurements were performed in front of the spanning tube and detector to obtain full spectra of high-sensitivity product ions. Due to these functional capabilities, biologically active substances that are part of the extracts of *A. tilesii* and *A. annua* were identified and evaluated (Fig. 3).

It was shown that the scanning speed of the mass spectrometer significantly affects the resolution of chromatography, and the scanning time of the whole mass spectrum should be less than the elution time of the chromatographic peak. It was found that one chromatographic peak of ethanol extract of “hairy” roots should have at least 5 complete mass spectra, which allows to obtain a spectrum with excellent statistical ions for reliable identification of compounds.

The *MassHunter Workstation* program was used to identify biologically active compounds by mass spectrum and to quantify their content even at very low concentrations (Table 1).

As it can be seen from table 1, extracts of *A. tilesii* and *A. annua* have a similar set of bioactive compounds, but they differed in the presence of some flavonoids and phenolic acids. Among phenolic acids: chlorogenic acid (peak 15), caffeoylquinic acid (peak 21), and gallic acid (peak 12) were presented both in *A. tilesii* and *A. annua* extracts (Table 1). Low amount of caffeic acid was observed only in *A. annua*. These results suggest that the distribution of phenolic acids in transgenic roots may be influenced by a variety of factors, including the nature of the “hairy” roots or extraction conditions.

Compounds found in ethanolic extracts of “hairy” roots have well-known medicinal properties. For example, antidiabetic and anti-inflammatory activity of sitosterol has been studied [22]. The positive effects of apigenin in the treatment of diabetes, neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease, depression, insomnia have been reported [23]. Antioxidant, anti-inflammatory, anti-amyloidogenic, and antitumor activity of named polyphenols were demonstrated [24].

High-performance liquid chromatography for the simultaneous quantification of

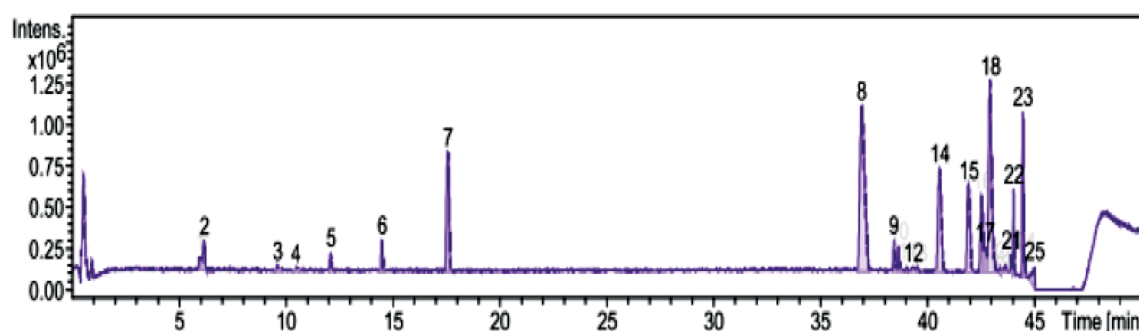

Fig. 3. **Representative chromatogram of ethanolic extract of *A. tilesii* "hairy" roots obtained by UPLC-ESI-UHR-Qq-TOF-MS (negative ion mode)**

Table 1. **Bioactive compounds in ethanolic extracts of *Artemisia tilesii* and *A. annua* "hairy" roots according to the UPLC-ESI-UHR-Qq-TOF-MS method (RT — retention time)**

Identified compound	Molecular ion in the MS spectra (m/z)	*A. tilesii*		*A. annua*	
		RT, min	Presence of compound	RT, min	Presence of compound
Caffeic acid	169.8956	–	–	38.7	+
Chlorogenic acid	353.2015	41.9	+	41.7	+
Caffeoylquinic acid	353.1438	43.9	+	43.9	+
Galic acid	170.0241	39.3	+	39.3	+
Quercetin, [M-H]-	300.1751	40.6	+	40.6	+
Luteolin-7-β-D-glucopyranoside	475.1245	14.5	+	14.5	+
Arginine	269.1486	36.9	+	37.0	+
Isorhamnetin 3-O-glucoside, [M+H]+	476.2794	38.4	+	–	–
Baikalein-7-O-glucuronide	445.1860	17.6	+	–	–
Sucrose, [M+K]+	381.1744	44.6	+	14.5	+
Sitosterol [M–H_2O]+	397.2278	42.5	+	42.7	+
Caffeoylshiqimic acids [M+H]+ Sterebin J/ Sterebin I, M+	327.1280	43.7	+	43.7	+
Kaempferol-3-O-galactoside-rhamnoside-7-O-rhamnoside	739.1577	45.0	+	–	–
Apigenin-7-O-glucoside	433.1140	12.1	+	11.9	+

biologically active components in extracts of "hairy" roots.

To quantify polyphenols and phenolic acids as components of the extracts of "hairy" roots, several chromatographic techniques described in the literature were tested [13, 15]. Generally, two mobile phases can be used in the reversed-phase HPLC polyphenol analyses for the separation of bioactive components in a mixture. However, the HPLC chromatogram of ethanol extract according to the well-known method for the determination of polyphenols [13] showed poor resolution of the chromatographic peaks of the detected substances (Fig. 4).

However, preference of the mobile phase in HPLC separation depends on the type and nature of the polyphenol compounds in the extract. HPLC techniques for the simultaneous determination of flavonoids and phenolic acids need to be improved to determine the quantitative composition of the components of ethanolic extracts of *A. tilesii* and *A. annua* "hairy" roots. Total fractions of extracts of "hairy" roots were previously purified from the solid phase. For comparison, the extract

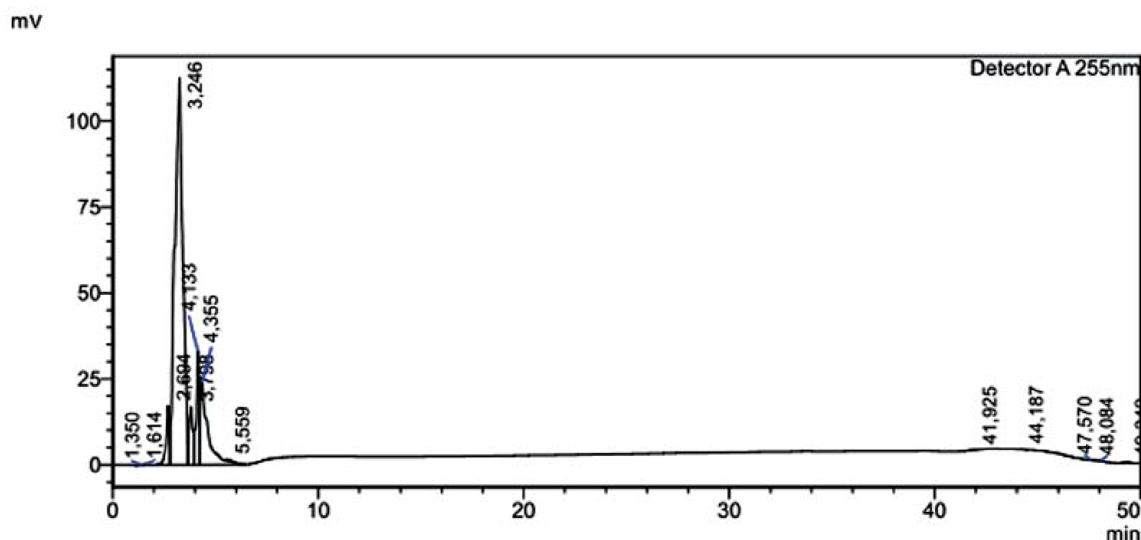

Fig. 4. **Chromatogram of the ethanol extract of "hairy" roots of *A. annua* obtained by the method described in [13]**

fraction and a mixture of polyphenol standards were chromatographed.

Optimization of extraction chromatography conditions in the determination of flavonoids and polyphenolic acids content.

Usually, the one mobile phase (Solvent A) contains water with organic acid (acetic acid, formic acid, and trifluoroacetic acid) and the other mobile phase (Solvent B) — pure organic solvents (methanol or acetonitrile). The organic solvents are necessary to reduce the chromatographic peak giving sharper peaks [13]. The ratio of methanol and water is very important when measuring isolated peaks of flavonoids by chromatography method. In this study, the effect of this parameter was evaluated using various conditions including methanol-water (10:90), acetonitrile-water (10:90), methanol-HCOOH, and other mobile phases.

We used the mobile phase containing 0.1–2% formic acid solution in different ratios CH_3OH/H_2O (Solvent A) and acetonitrile (Solvent B). In the study the flow rate was adjusted in the range of 0.5–1.0 ml/min, the column was thermostated at 25–35 °C, and the volume for other injections ranged from 10 to 30 µl. Gradient elution was performed by changing the proportion of solvent B to solvent A. The gradient was changed from 10% to 40% B linearly for 28 minutes, from 40 to 60% B for 39 min, from 60 to 90% B for 50 min. The mobile phase composition was returned to its original state (solvent B:solvent A — 10:90) after 55 min and allowed to work for another 10 min before the introduction of another sample. The total analysis time of the sample was 65 min. Chromatograms were detected by HPLC-DAD at three different wavelengths ranging from 200 to 300 nm, which allowed maximum absorption, improved the resolution, and smoothed the baseline. The analysis was performed with or without a pre-column.

It should be noted that flavonoids, which are relatively less polar, were found mainly in less polar media, and their concentration is somewhat increased in polar media, given the synergistic effect of the components present in the extract, which restricts the freedom of OH-groups in the complex biomatrix. On the contrary, the increased influence of the polarity of the solvent together with the concomitant cleavage of the ether bond led to the improved separation of phenolic acids. The use of methanol with different ratios to water as a mobile phase was the compromise approach. It was found that the best ratio of methanol/water is 25/75 (v/v), which allowed to separate the maximum number of components in the mixture, as well as to quantify them. Optimization of the chromatographic conditions of the extract in gradient mode using methanol improved the separation of bioactive components, as shown in Fig. 5.

After optimization of the chromatography conditions, the extracts were analyzed in gradient mode using the following solvent system: mobile phase *A*: 0.1% (v/v) formic acid in methanol/water (25/75, v/v); mobile phase *B*: acetonitrile. The absorption spectra were recorded at 255 nm. The column temperature was 28 °C and the injection volume was 20 µl.

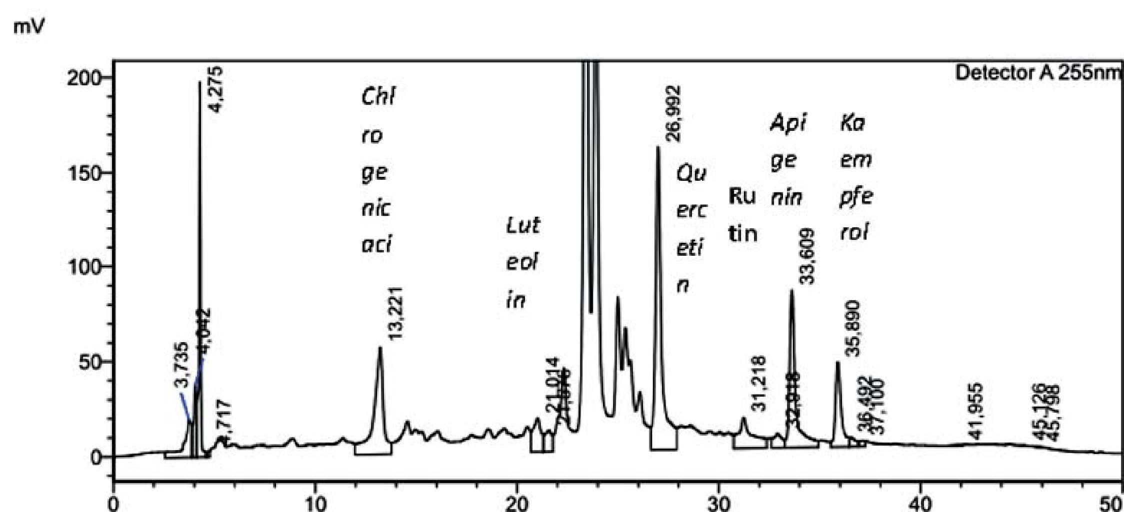

Fig. 5. **Chromatogram of ethanolic extract of *A. annua* "hairy" roots under optimized chromatographic conditions**

Validation of the method of quantitative determination of phenolic acids and flavonoids. The quantitative content of flavonoids and phenolic acids was determined using an external standard by constructing calibration curves for six representative polyphenolic compounds of the appropriate classes (chlorogenic acid, apigenin, luteolin, quercetin, kaempferol, and rutin).

Metrological parameters of the developed chromatographic method for the determination of principal components in ethanolic extracts of *A. tilesii* and *A. annua* "hairy" roots are given in Table 3.

The developed chromatographic method for the determination of biologically active compounds showed good separation of components in the mixture (Fig. 5). The linearity of the developed method was determined with the reference standard solutions with the concentration range of 0.025–100.00 mg/L at five concentration levels with correlation coefficients R2 from 0.9997 to 0.9999 (Table 3). LOD for six studied bioactive compounds ranged from 2.73 to 4.95 ng/mL, while LOQ ranged from 9.11 to 16.51 ng/mL, which is the evidence of the sensitivity of the used procedure. High values of recovery (in the range of 97.27–98.95%) indicated the correctness of the developed methodology. Therefore, studies of validation parameters of the method confirmed the validity of quantitative analysis of detectable polyphenolic compounds.

Ethanol extracts of *A. tilesii* and *A. annua* "hairy" roots were analyzed using the developed method (Table 4).

Biologically active compounds were detected in samples of extracts at concentrations of 0–0.282 mg/ml. The presence of Apigenin (0.168 ± 0.003 mg/ml and 0.178 ± 0.006 mg/ml), Quercetin (0.282 ± 0.005 mg/l and 0.174 ± 0.005 mg/ml) in the extracts of *A. annua* and *A. tilesii* was shown. It was found that the "hairy" roots of plants of two species of *Artemisia* genus differed in

Table 3. **Metrological parameters of the developed chromatographic method for determination of flavonoids and phenolic acids in ethanolic extracts of *A. tilesii* and *A. annua* "hairy" roots**

Component	Linearity, mg/L	Calibration curves	R_2	LOD, ng/mL	LOQ, ng/mL	Recovery, %
Chlorogenic acid	0.025–100.00	y = 4538.6x + 5.0975	0.9997	3.19	10.63	96.05
Apigenin		y = 1053.6x +0.5834	0.9997	4.95	16.51	98.76
Luteolin		y = 7808.1x + 9.7779	0.9998	3.28	10.94	97.45
Rutin		y = 2405.1x + 3.2862	0.9997	4.62	15.41	98.01
Quercetin		y = 6019.6x + 7.6469	0.9997	3.49	11.63	97.27
Kempferol		y = 5899.4x + 9.4264	0.9999	2.73	9.11	98.95

Table 4. **Quantification of phenolic acids and flavonoids (mg/ml) in ethanolic extracts of *Artemisia tilesii* and *A. annua* “hairy” roots**

No	Compound	Content in *A. annua*	RSD, %	Content in *A. tilesii*	RSD, %
1	Chlorogenic acid	0.192 ± 0.004	1.04	0.107 ± 0.005	1.10
2	Apigenin	0.168 ± 0.003	0.98	0.178 ± 0.006	1.12
3	Luteolin	0.037 ± 0.003	0.95	0.112 ± 0.004	0.96
4	Quercetin	0.282 ± 0.005	1.11	0.174 ± 0.005	1.10
5	Rutin	0.075 ± 0.005	1.10	0.056 ± 0.007	1.11
6	Kaempferol	0.108 ± 0.003	0.96	0.142 ± 0.003	0.95

polyphenolic compounds concentration. The study indicated the presence of high amounts of chlorogenic acid and quercetin in extracts of *A. annua* roots, while *A. tilesii* roots had a higher content of luteolin and kaempferol (Table 4). In a complex multicomponent extract, chlorogenic acid remains either in the free state or in combination in the form of ether and acts as a powerful antioxidant. Stability tests showed that the extracts of “hairy” roots were stable for 2 months. The relative standard deviation (RSD) of polyphenols was from 0.95 to 1.12% , respectively, indicating a good precision of measurements.

The presence of the determined flavonoids and phenolic acids was linked to certain positive health effects and other bioactive functions that have already been reported in the literature to highlight the potential functional activity of the analyzed “hairy” roots [4, 6, 7].

Conclusions

Thus, the profile of polyphenolic compounds in extracts from *A. tilesii* and *A. annua* “hairy” roots was studied. The total content of polyphenols was used as a control parameter for the efficiency of the extracts study. The identification of a significant amount of valuable biologically active components in the studied wormwood “hairy” roots and variation in the amount determined based on the polarity of the solvent taken for the extraction process provides a clear recommendation to use the proposed method of extraction and determining the content of polyphenolic compounds in extracts in pharmacy and medical practice. The proposed UPLC-ESI-UHR-Qq-TOF-MS method was studied as a useful tool for the rapid detection and structural characterization of polyphenolic compounds in complex matrix such as ethanolic extract. Six flavonoid glycosides, 2 non-glycosylated flavonoids with several phenolic acids were detected in ethanolic extracts of *A. tilesii* and *A. annua* “hairy” roots.

The reversed-phase HPLC-DAD technique has been developed to quantify phenolic acids and flavonoids in ethanolic extracts of *A. tilesii* and *A. annua* “hairy” roots. The developed HPLC method for the determination of biologically active compounds showed good separation of components in the plant extracts. It was characterized by a wide range of linearity, was sensitive and accurate. So, the method is suitable for the simultaneous determination of phenolic acids and flavonoids with optimal analysis time and high efficiency. The developed technique can be used for the analysis of extracts of different *Artemisia* species and in the preparation of monographs for the pharmacopoeia.

This work was partially supported by the Ukrainian-Bilorusian grant (2021-2022, No. M/103-2021) from the Ministry of Education and Science of Ukraine.

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ОДНОЧАСНА ІДЕНТИФІКАЦІЯ, КІЛЬКІСНЕ ВИЗНАЧЕННЯ ТА АНАЛІЗ ОСНОВНИХ КОМПОНЕНТІВ ЕКСТРАКТІВ «БОРОДАТИХ» КОРЕНІВ *Artemisia annua* ТА *Artemisia tilesii*

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Мета — дослідження профілів поліфенольних сполук в екстрактах «бородатих» коренів *Artemisia tilesii* Ledeb. і *A. annua* L., аналітичне розділення і кількісне визначення компонентів.

Методи. «Бородаті» корені вирощували *in vitro* на середовищі Мурасіге і Скуга. Високоефективна хроматографія з різними видами детектування (тандемним квадруполь-часопролітним мас-спектрометром та діодно-матричним детектуванням) було використано для ідентифікації та кількісного визначення основних компонентів у складі етанольних екстрактів «бородатих» коренів.

Результати. У більшості зразків «бородатих» коренів вміст флавоноїдів був вищим за вміст у коренях контрольних рослин. Показано наявність в етанольних екстрактах «бородатих» коренів *A. annua* та *A. tilesii* апігеніна (0,168 ± 0,003 мг/л і 0,178 ± 0,006 мг/л), кверцетина (0,282 ± 0,005 мг/л і 0,174 ± 0,005 мг/л), виявлено хлорогенову кислоту, кемпферол та інші флавоноїди.

Висновки. Розроблено методику ВЕРХ з діодно-матричним детектуванням для кількісного визначення основних компонентів в екстрактах «бородатих» коренів, що характеризується широким діапазоном лінійності, високою чутливістю (9,11 нг/мл ≤ LOQ ≤ 16,51 нг/мл), точністю та коректністю. Методика дозволяє робити одночасне визначення фенольних кислот та флавоноїдів з оптимальним часом і високою ефективністю. Розроблена методика може бути використана для аналізу екстрактів рослин різних видів.

Ключові слова: *Artemisia tilesii, Artemisia annua,* поліфеноли, флавоноїди, «бородаті» корені, обернено-фазова ВЕРХ із діодним матричним детектором.

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