

ANALYSIS OF PHENOLIC COMPOUNDS AND BIOLOGICAL ACTIVITY OF PLANTS OF THE GENUS CLIMACOPTERA BRACHIATA

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Abstract. *Climacoptera brachiata* is an annual species of halophytes with great potential for use in urban landscapes. Extracts of *Climacoptera brachiata* have been used in traditional medicine for many years due to their antifungal, antibacterial activity due to various biologically active compounds. It is known that the leaves contain triterpenoids, and the flowers contain phenolic compounds. The authors proposed a scheme for the analysis of water-alcohol extracts of *Climacoptera brachiata*. In addition, extracts were screened and determined by high-performance liquid chromatography. For the simultaneous analysis of phenolic compounds, a fast and sensitive method for the determination of natural compounds using high-performance reverse phase liquid chromatography (RP-HPLC) in combination with a diode matrix detector (DAD) has been developed. Gradient elution systems of 0.1% acetic acid and methanol were linearly used, the analysis time was 57 minutes. The method was confirmed by linearity, relative error, reproducibility, and LOD values. The detection of compounds was carried out at 254 nm. The developed method for the rapid determination of phenolic compounds using RP-HPLC was used to determine the presence of phenolic compounds in natural and commercial products.

Keywords: phenolic compounds, antioxidant activity, antidiabetic inhibitory activity, HPLC.

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1. Introduction

Kazakhstan, the area of growth of many types of plant objects, the study of their chemical composition and the screening of biologically active substances require further research. At the present stage, the creation and development of phytopreparations is a promising and alternative direction to replace synthetic drugs with a number of side effects. Since synthetic drugs have many side effects, researchers are beginning to study natural products in order to find effective medicines. Therefore, the study of domestic wild plants and the introduction of new phytopreparations from them into industry is a timely task. One of the most relevant ways to obtain new biologically active substances is to isolate compounds from plants. Consequently, on this path it is necessary to solve complex problems of isolation and deep purification from complex multicomponent biological objects of sufficiently labile target substances, unambiguous identification of structures.

Currently, plants of the Chenopodiaceae family, the genus *Climacoptera*, which are widespread in Kazakhstan and some of them are endemic plants, are of great theoretical and practical interest. [1-2] and so, the search for new sources of biologically active compounds among plants of the Chenopodiaceae family growing in arid zones has not been exhausted.

In this regard, the study of the chemical composition of new plant species, the development of a flowchart for the isolation of biologically active compounds, the establishment of their biological activity and the development of new phytopreparations from Kazakhstani wild plant species has not only a socially significant effect, but also makes it possible to expand the range of medicines from local wild-growing renewable sources of plant material [3]. Development of chromatographic identification and analysis methods based on chemical components, in particular phenolic compounds, can contribute to the standardization of medicinal raw materials and extracts.

2. The experimental part

The object of our research is the aboveground part of plants of the genus *Climacoptera*, the species *Climacoptera brachiata*, collected during the flowering phase in Ili district, Almaty region in 2023.

Conditions for HPLC analysis for the determination of phenolic compounds. The analysis of phenolic compounds was determined using the Shimadzu high performance liquid chromatography system (Shimadzu Cooperation, Japan), which consists of a Shimadzu solvent supply unit model LC-20AT and a Shimadzu diode array detection system model SPD-M20A. All parts are controlled using LC-solution software (CBM-20A Shimadzu system controller). The temperature in the column is set at 35°C. Chromatographic separation was performed on an Inertsil ODS-3 column (4 microns, 4.0 mm x 150 mm) and in an Inertsil ODS-3 protective column. The mobile phase is 0.1% acetic acid in water (A) and 0.1% acetic acid in methanol (B). The elution profile was as follows: 2% B: for 3 min, 2-5% In; for 3 minutes, 5-6% In; for 2 minutes, 6-10% In; for 4 min,

10% In; for 1 min, 10-25% In; for 5 minutes, 25-30% In; for 7 minutes, 30-40% B; for 5 minutes, 40-42% B; for 6 minutes, 42-54% B; for 5 min, 54-55% B; for 1 min, 55-56% B; for 10 min, 56-65% B; for 4 min, 65-75% B; for 3 minutes, 75-85% B; for 2 minutes, 85-95% B; for 5 minutes, 95% B; for 2 minutes, 95-100% B; for 1 minute, 100% B; 5 minutes, 100-80% B; 2 minutes, 80-50% B; in 2 minutes, 50-2% B. The flow rate was 1.0 ml/min. The injection volume is 20 µl. The detection was carried out using a diode matrix detector (DAD) using wavelengths of 200-600 nm. All samples and standards were filtered using an Agilent PTFE filter with a thickness of 0.45 microns [4].

Determination of antioxidant activity. To determine the antioxidant activity, four methods were used: ABTS methods for removing cation radicals and DPPH free radicals, as well as methods for reducing the antioxidant capacity of copper (CUPRAC) and β-carotene/linoleic acid discoloration.

Analysis for discoloration by β-carotene/linoleic acid. The overall antioxidant activity was assessed using the beta-carotene/linoleic acid test system with minor changes. β-carotene (0.5 mg) diluted in 1 ml of chloroform is added to 25 ml of linoleic acid and 200 mg of a mixture of Tween 40 emulsifiers. After evaporation of chloroform under vacuum with intensive shaking, 100 ml of distilled water saturated with oxygen is added. 160 µl of this mixture is added to 40 µl of samples of various concentrations. Once the emulsion is added to each tube, the absorption at time zero is measured at a wavelength of 470 nm using a 96-well microplate reader [5]. After incubating the tablet for 2 hours at 50 °C, the absorption of the emulsion is measured again at the same wavelength. Ethanol is used as a control. BHA and α-tocopherol are used as antioxidant standards for activity comparison. The degree of discoloration (R) of β-carotene is calculated according to the following equation:

$$R = \frac{\ln \frac{a}{b}}{t}$$

where ln is the natural logarithm, a is the absorption at time zero and b is the absorption at time t (120 min). The calculation of antioxidant activity is carried out as a percentage of inhibition compared to the control using the following equation:

$$\text{Antioxidant activity(\%)} = \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \times 100$$

Analysis for the removal of free radicals by the DPPH method. The activity of removing free radicals is determined spectrophotometrically using the DPPH method described by Blois [6], with minor modifications. In its radical form, DPPH absorbs at a wavelength of 517 nm, but when reduced by an antioxidant or a radical substance, its absorption decreases. In short, 120 ml of ethanol and 40 µl of the sample solution dissolved in an aqueous alcohol solution in various

concentrations are mixed. The reaction is then initiated by adding 0.4 mm 40 μ l of DPPH prepared in ethanol. After thirty minutes, the absorption is measured at a wavelength of 517 nm using a 96-well microplate. Ethanol is used as a control. A lower absorption of the reaction mixture indicates a higher free radical scavenging activity. The ability to trap DPPH radicals is calculated using the following equation:

$$\text{Absorbing effect DPPH (\%)} = \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \times 100$$

ABTS analysis for cationic radical discoloration. The spectrophotometric analysis of the absorbing activity of $\text{ABTS}^{\cdot+}$ was carried out in accordance with the method [7], with minor changes. $\text{ABTS}^{\cdot+}$ was obtained as a result of a reaction between 7 m ABTS in H_2O and 2.45 m potassium persulfate, which was stored in the dark at room temperature for 12 hours. The radical cation was stable in this form for more than 2 days when stored in the dark at room temperature. Before use, the $\text{ABTS}^{\cdot+}$ solution is diluted with ethanol to obtain an optical density of 0.708 ± 0.025 at 734 nm. Then, 160 ml of $\text{ABTS}^{\cdot+}$ solution in various concentrations is added to 40 ml of the sample solution in ethanol. After 10 minutes, using a 96-well microplate reader, the percentage of inhibition is calculated at a wavelength of 734 nm for each concentration of relatively pure absorption (ethanol). The following equation is used to calculate the absorption capacity of $\text{ABTS}^{\cdot+}$:

$$\text{Absorbing effect ABTS (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Antioxidant ability that reduces copper content. The antioxidant capacity, which reduces the copper content, was determined in accordance with the method [8], with minor changes. 50 μ l of 10 mm copper (II), 50 μ l of 7.5 mm neocuproine and 60 μ l of NH_4Ac buffer (1 M, (pH=7.0)) are added to each well in a 96-well plate. 40 ml of extract is added to the initial mixture in various concentrations so that the final volume is 200 ml. After 1 hour, the absorption at a wavelength of 450 nm was recorded on the reagent blank using a 96-well microplate reader. The results are presented in the form of an absorption coefficient and compared with the indicators of the standard used as an antioxidant standard [9].

Antidiabetic inhibitory activity. Antidiabetic inhibitory activity was determined by the inhibitory activities of α -amylase and α -glucosidase spectrophotometrically using a 96-well microplate reader [10,11]. Ethanol was used as a control. Acarbose has been used as an antidiabetic inhibitory standard to compare activity.

3. Discussion of the results

Using the method of ultrasonic extraction and maceration, 10 samples of extracts from plants of the genus *Climacoptera brachiata* were obtained.

According to the requirements of the Government Pharmacopoeia, the quality of the raw materials was determined: moisture 6.02%, ash content 9.6%, the amount of extractive substances 21.3% in 50% water alcohol, the extract is concentrated and the dry extract is preserved. To evaluate the antioxidant and antidiabetic inhibitory activity, and to determine phenolic compounds, a 50% water-alcohol extract of a plant of the genus *Climacoptera brachiata* was used.

Table 1 - Phenolic compounds of 50% water-alcohol extract of *Climacoptera brachiata* plant by HPLC-DAD method (mg/g)

The phenolic compound	RT	calib. eq.	R ²	<i>Climacoptera</i>
Fumaric acid	14,014	$y = 1988.9x - 4655.8$	0.9998	1.90
Protocatechuic acid	24,625	$y = 65753x - 6932.1$	0.9999	0.15
Pyrocatechol	24,658	$y = 3772.8x + 23692$	0.9925	-
Theobromine	25,967	$y = 3942.7x + 81451$	0.9983	<i>tr</i>
Theophylline	29,449	$y = 36694x + 68674$	0.9998	-
4-hydroxybenzoic acid	30,867	$y = 123758x + 75779$	0.9997	<i>tr</i>
4-hydroxybenzaldehyde	33,367	$y = 34376x + 4239.6$	0.9996	-
Vanillic acid	34,758	$y = 66764x + 46508$	0.9998	<i>tr</i>
Epicatechin	35,278	$y = 2097.6x + 7998.2$	0.998	-
Caffeic acid	35.28	$y = 49533x + 213471$	0.9957	<i>tr</i>
Vanillin	36,915	$y = 21426x + 195103$	0.9995	<i>tr</i>
Chlorogenic acid	40,094	$y = 46920x - 36953$	0.9995	0.10
Coupe -coumaric acid	40,874	$y = 17265x + 343183$	0.9951	<i>tr</i>
Ferulic acid	42,564	$y = 42245x + 110701$	0.9992	<i>tr</i>
Coumarin	45,178	$y = 81802x + 153471$	0.9968	<i>tr</i>
Propyl gallate	46,984	$y = 29731x - 12781$	1	-
Routine	47,527	$y = 47899x + 56096$	0.9997	<i>tr</i>
<i>trans</i> -2-OH cinnamic acid	48,243	$y = 53442x + 104662$	0.9996	-
Fisetin	51,243	$y = 100784x + 16688$	0.9984	0.49
<i>trans</i> - Cinnamic acid	56,203	$y = 88190x + 158733$	0.9997	<i>tr</i>
Genistein	57,739	$y = 69160x + 5753.8$	0.9996	<i>tr</i>

tr: number of tracks;

- not detected.

To determine optimal chromatographic results, experiments were conducted under various mobile phase conditions. In order to obtain the best possible resolution, the mobile phases of acetonitrile, methanol, acetonitrile-acetic acid and methanol-acetic acid were used by changing the ratio of acetic acid. The best

mobile phase for separation was 0.1% acetic acid in water as solvent A and methanol as solvent B. Many studies have suggested the use of acetonitrile as one of the mobile phases. Acetonitrile was also used for separation. However, only 4 compounds were isolated, namely fumaric acid (1.90 mg/g), protocatechic acid (0.15 mg/g), chlorogenic acid (0.10 mg/g), and fisetin (0.49 mg/g). Therefore, the goal was to change the solvent system to simultaneously analyze more phenolic components. For this purpose, Inertsil ODS-3 (4 microns, 4.0 mm) was used to obtain clear peaks and reduce the elution time. An analytical column with a diameter of 150 mm was used. The column temperature was maintained at 35°C. Using this optimized method, phenolic components were determined with high accuracy within 57 minutes.

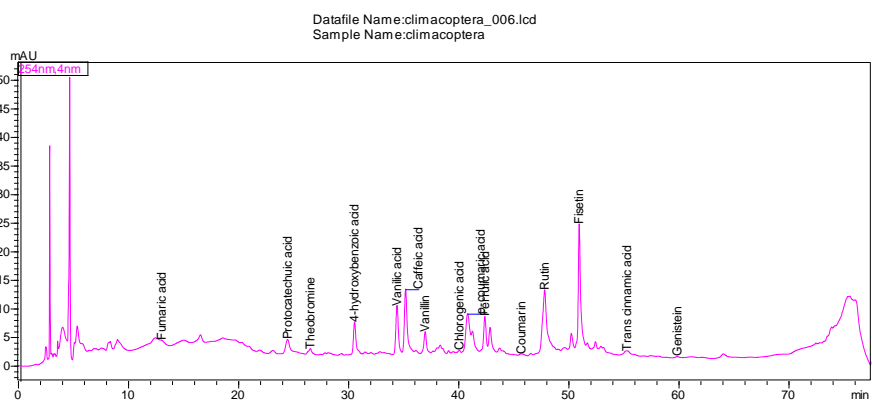


Figure 1 - HPLC–DAD chromatogram of *Climacoptera* at 254 nm (Inertsil ODS-3 column (4 μ m, 4 mm x 150 mm). Mobile phase 0.1% acetic acid-methanol (gradient elution). Flow rate 1mL/min. Diode array detection 254 nm.).

The antidiabetic inhibitory activity of 50% water alcohol extract was evaluated by the inhibitory activities of α -amylase and α -glucosidase, and the standard acarbose was used as the antidiabetic inhibitory activity. The data obtained is shown in table 2.

Table 2 - Antidiabetic inhibitory activity of 50% water-alcohol extract of a plant of the genus *Climacoptera brachiata*.

Sample	Antidiabetic inhibitory activity	
	inhibitory activity of α -amylase IC ₅₀ (μ g/mL)	inhibitory activity of α -glucosidase IC ₅₀ (μ g/mL)
<i>Climacoptera</i>	92985.20 \pm 0.50	172.84 \pm 3.43
Acarbose ^b	36.74 \pm 4.50	22.28 \pm 2.03

^a The values shown here are the average \pm SEM based on the results of three parallel measurements. p<0,05.

^breference compounds.

Table 3 - Antioxidant activity of 50% water-alcohol extract of a plant of the genus *Climacoptera*, brachiata.

Sample	Antioxidant activity			
	ABTS ⁺ IC ₅₀ (µg/mL)	DPPH IC ₅₀ (µg/mL)	CUPRAC IC ₅₀ (µg/mL)	β-Carotene/linoleic acid assay IC ₅₀ (µg/mL)
<i>Climacoptera</i>	65.12±6.97 (50-400 µg/mL)	474.46±0.65 (50-400 µg/mL)	226.35±0.09 (50-400 µg/mL)	184.87±0.23 (25-200 µg/mL)
BHA ^b	1.50 ± 0.14	4.36 ± 0.31	4.94 ± 0.08	2.48 ± 0.64
α-TOC ^b				2.21±0.77

^a The values shown here are the average ± SEM based on the results of three parallel measurements. p<0,05.

^breference compounds.

The table shows the results of antioxidant activity by ABTS methods for removing cation radicals and DPPH free radicals, as well as methods for reducing the antioxidant capacity of copper (CUPRAC) and discoloration with beta-carotene/linoleic acid. BHA (butylhydroxyanisole) was used as an antioxidant standard for activity comparison, and alpha-tocopherol was also used to determine the activity of beta-carotene/linoleic acid discoloration.

4. Conclusions

For the first time, the RP-HPLC-DAD method in this study demonstrated the content of 4 phenolic compounds from *Climacoptera brachiata* plants, which can be useful as a chemical marker for quality control of plant raw materials. A practical, fast and accurate RP-HPLC-DAD method has been developed for simultaneous analysis of natural compounds in one injection. The total analysis time was 57 minutes. A wavelength of 254 nm was used to determine the compounds with high accuracy.

Some of the natural products studied are potential medicinal products because they have sufficient antidiabetic inhibitory activity (α-amylase inhibitory activity 92985.20±0.50 and IC₅₀ (µg/mL) alpha-glucosidase inhibitory activity IC₅₀ (µg/mL) 172.84±3.43)

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Conflict of Interest: The authors declare that there is no conflict of interest requiring disclosure in this article

CLIMACOPTERA BRACHIATA ТЕКТІ ӨСІМДІКТЕРІНІҢ ФЕНОЛДЫҚ ҚОСЫЛЫСТАРЫ МЕН БИОЛОГИЯЛЫҚ БЕЛСЕНДІЛІГІН ТАЛДАУ

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Түйіндеме: *Climacoptera brachiata* - қалалық ландшафттарда пайдалану үшін үлкен әлеуеті бар галофиттердің бір жылдық түрі. Сығындылары дәстүрлі медицинада көптеген жылдар бойы қолданылады, қасиеттеріне сүйене отырып тұмауға қарсы, бактерияға қарсы белсенділік, олар әртүрлі биологиялық белсенді қосылыстардан туындайды. Жапырақтарда тритерпеноидтар, ал гүлдерде фенолды қосылыс болатыны белгілі. Авторлар қсыныстары бойынша сығындылардың талдау схемасын әзірледі. Сонымен қатар, сығындылардың биоскринингі және жоғары тиімді сұйық хроматография әдісімен анықтау жүргізілді. Фенолдық қосылыстарды бір мезгілде талдау үшін диодты матрицалық детектормен (DAD) біріктірілген жоғары тиімді кері фазалы сұйық хроматографияны (RP-HPLC) пайдалана отырып, табиғи қосылыстарды анықтаудың жылдам және сезімтал әдісі әзірленді. 0,1% сірке қышқылы мен метанолдың градиентті элюция жүйелері сызықтық түрде қолданылды, талдау уақыты 57 минутты құрады. Әдіс сызықтық, салыстырмалы қателік, қайталану, LOD мәндерімен расталды. Қосылыстарды анықтау 254 нм - де жүргізілді. RP-HPLC көмегімен фенолдық қосылыстарды жылдам анықтаудың әзірленген әдісі табиғи және коммерциялық өнімдерде фенолдық қосылыстардың болуын анықтау үшін қолданылады.

Түйін сөздер: фенолды қосылыстар, тотығуға үрдісіне қарсы белсенділік, диабетке қарсы ингибиторлық белсенділік, HPLC.

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АНАЛИЗ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ РАСТЕНИЙ РОДА CLIMACOPTERA BRACHIATA

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Резюме: *Climacoptera brachiata* (*К. супротивнолистая*) - однолетний вид галофитов, обладающий большим потенциалом для использования в городских ландшафтах. Экстракты *Climacoptera brachiata* используются в традиционной медицине в течение многих лет, благодаря их противогрипковой, антибактериальной активности, которые обусловлены разными биологически активными соединениями. Известно, что в листьях содержатся тритерпеноиды, а в цветках фенольные соединения. Авторами предложена схема анализа водно-спиртовых экстрактов. Кроме того, проведен биоскрининг экстрактов и определение методом высокоэффективной жидкостной хроматографии. Для одновременного анализа фенольных соединений разработан быстрый и чувствительный метод определения природных соединений с использованием высокоэффективной жидкостной хроматографии с обратной фазой (RP-ВЭЖХ) в сочетании с диодным матричным детектором (DAD). Линейно использованы системы градиентного элюирования 0,1% уксусной кислоты и метанола, время анализа составило 57 минут. Метод был подтвержден линейностью, относительной погрешностью, во воспроизводимостью, значениями LOD. Обнаружение соединений проведены при 254 нм. Разработанный метод быстрого определения фенольных соединений с помощью RP-ВЭЖХ использован, для определения наличия фенольных соединений в природных и коммерческих продуктах.

Ключевые слова: фенольные соединения, антиоксидантная активность, антидиабетическая активность, ингибирующая активность, ВЭЖХ.

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