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Разработка показателей качества сырья «Череды поникшей трава»

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Аннотация. ***Введение.*** Череда поникшая (Bidens cernua L.) является перспективным производящим лекарственным растением, содержащим флавоноиды и полиацетилены, однако оно недостаточно изучено и не имеет установленных фармакопейных стандартов качества на получаемое из него лекарственное растительное сырьё. ***Цель.*** Разработка комплекса нормируемых показателей качества для «Череды поникшей травы», включая макро- и микроскопические признаки, числовые нормативы и методы хроматографической идентификации, для её стандартизации и потенциального использования в фармацевтике и производстве БАД. ***Материалы и методы.*** Исследование проводили на образцах надземной части череды поникшей, собранных в Московской области и Ставропольском крае. Применяли стандартные фармакопейные методики (ГФ РФ XIV изд.) для определения морфологических, микроскопических признаков и числовых показателей (влажность, зольность, примеси, экстрактивные вещества). Для качественного анализа полиацетиленов и флавоноидов использовали тонкослойную хроматографию (ТСХ). ***Результаты и обсуждение.*** Установлены диагностические макро- и микроскопические признаки сырья. На основе экспериментальных данных предложены следующие нормы: влажность – не более 12%; общая зола – не более 7%; зола, нерастворимая в 10% HCl – не более 2%; органическая примесь – не более 2%; минеральная примесь – не более 1%; экстрактивные вещества (70% этанол) – не менее 30%. Разработаны и валидированы методики ТСХ-идентификации полиацетиленов и флавоноидов для подтверждения подлинности сырья. ***Заключение.*** Разработанный комплекс показателей качества позволяет стандартизировать сырьё «Череды поникшей трава». Полученные результаты обосновывают его перспективность для создания БАД с антиоксидантной, капилляроукрепляющей, противовоспалительной и дерматотропной активностью.

Ключевые слова: череда поникшая, полиацетиленовые соединения, флавоноиды, показатели подлинности и качества

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Research article

Development of quality indicators for the raw material "Bidentis cenuae herba"

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Abstract. ***Introduction.*** Bidens cernua L. is a promising medicinal plant producing flavonoids and polyacetylenes; however, it has been insufficiently studied and has no established pharmacopoeial quality standards for the medicinal plant materials obtained from it. ***Goal.*** To develop a set of standardized quality indicators for "Bidentis cernuae herba", including macro- and microscopic characteristics, numerical standards, and chromatographic identification methods, for its standardization and potential use in pharmaceuticals and dietary supplement production. ***Materials and Methods.*** The study was conducted on samples of the aerial parts of Bidens cernua collected in the Moscow Region and Stavropol Krai. Standard pharmacopoeial methods (State Pharmacopoeia of the Russian Federation, 14th edition) were used to determine morphological, microscopic characteristics, and numerical indicators (moisture, ash content, impurities, extractives). Thin-layer chromatography (TLC) was used for the qualitative analysis of polyacetylenes and flavonoids. ***Results and discussion.*** Diagnostic macro- and microscopic properties of the raw material were established. Based on the experimental data, the following standards were proposed: moisture – no more than 12%; total ash – no more than 7%; ash insoluble in 10% HCl – no more than 2%; organic impurity – no more than 2%; mineral impurity – no more than 1%; extractive substances (70% ethanol) – no less than 30%. TLC identification methods for polyacetylenes and flavonoids were developed and validated to confirm the authenticity of the raw material. ***Conclusion.*** The developed set of quality indicators allows standardizing the raw material of "Bidentis cernuae herba". The obtained results substantiate its potential for the creation of a dietary supplement with antioxidant, capillary-strengthening, anti-inflammatory and dermatotropic activity

Key words: Bidens cernua, polyacetylene compounds, flavonoids, indicators of authenticity and quality
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Introduction. Bidens cernua L. is a promising type of medicinal plant material containing a complex of biologically active compounds, in particular flavonoids and polyacetylenes, which determine its pharmacological activity [1-4]. Unlike Bidens tripartita L., the raw material of Bidens cernua has been studied to a much lesser extent and does not have established pharmacopoeial quality standards. The development of a set of indicators, including macro- and microscopic characteristics, as well as numerical indicators (moisture, ash content, impurity and extractive substance content), is a necessary step for the standardization of this type of raw material and ensuring its proper quality. Chromatographic determination of key groups of biologically active compounds serves as an important tool for identifying the raw material and confirming its authenticity. The present work is aimed at a comprehensive study and establishment of standardized characteristics for Bidens cernua L. herb, which will facilitate its

possible introduction into the composition of dietary supplements, in medical practice and pharmaceutical production.

Materials and methods of research. The object of the study were samples of the above-ground part of Bidens, drooping (upper 15-20 cm), collected during the flowering phase in the Moscow region and Stavropol territory. After collection, the samples were air-dried at a temperature not exceeding 35-40 °C. They were stored in paper bags in accordance with the requirements of OFS.1.1.0011.15 of the State Pharmacopoeia of the Russian Federation, XIV edition [5]. The study of external, microscopic features, quality indicators and the establishment of their standards for the studied raw materials were carried out using standard pharmacopoeial methods. For chromatographic analysis, Sorbfil PTSKh-P-A plates (10x15 and 10x10 cm) were used, activated for 60 minutes in a drying oven (100-105 ºC). Solutions of individual compounds, extracts and their fractions were applied by a standard method using a microsyringe.

Research results and discussion. As a result of the study, external characteristics were established for whole and crushed raw materials. “Whole raw materials consist of whole or partially crushed leafy shoots, their individual fragments (stems, leaves, flower baskets, unripe fruits). The leaves are simple, opposite, without a petiole, lanceolate up to 12 cm long. The apex is pointed, the margin is serrate-dentate. Flower baskets are 1.0–1.5 cm in diameter, collected in inflorescences on elongated stalks; bisexual, tubular. Involucral leaflets are linear-oblong, with spiny cilia along the margin. Achenes are inversely cuneate , with two (rarely 3-4) awns, half the length of the achene. The leaves are green or brownish-green, the stems are green, and the flowers are yellow or golden-yellow. The smell is faint and characteristic, and the taste is bitter.

"The crushed raw material (no more than 7 mm) is a mixture of fragments of flowers, buds, achenes, leaves, and stems, containing pieces of leaves, ribbed stems, flower heads, buds, and achenes of the corresponding color. The odor is faint, the taste is bitter."

Microscopic analysis revealed the following diagnostically significant structures:

– on the surface of the leaf blade, on both sides, anomocytic stomata and thin-walled hairs are found, consisting of 5-10 cells and resembling a caterpillar in shape;

– in the mesophyll of the leaf, especially along the veins and along its edge, numerous secretory passages of various shapes and thick-walled hairs formed by 4-6 cells are localized;

- on the surface of the epidermis of the outer and inner leaflets of the wrapper, cells with sinuous walls were found on both sides, as well as two types of hairs: caterpillar-like (of 6-10 cells) and thick-walled (of 4-6 cells);

– when examining the petals of the corolla of tubular flowers under a microscope, especially at their base, secretory passages, caterpillar-like hairs and pollen grains with a spiny surface are observed.

Determination of quality indicators and standards. Moisture determination. The moisture content of the raw materials was determined in accordance with the methodology of the State Pharmacopoeia of the Russian Federation, 14th edition [5]. The results are presented in Table 1.

Table 1 – Results of determining the moisture content of the Chamaenerion angustifolium herb a

Collection region raw materials	f	Humidity value, % (x)	$\bar{x}$	S	t_P(f)	Δx, %	E, %
Stavropol Krai	5	11.05; 11.44; 11.25; 10.17; 10.96; 11.12	11:00	0.4390	2.57	0.4606	4.19
Moscow region	5	11.19; 10.82; 10.76; 10.35; 11.48; 10.61	10.87	0.4067	2.57	0.4267	3.93

Experimental data demonstrate that the moisture content of raw materials from the Stavropol Krai ranges from 10.17% to 11.44%, with an average of 11.00%. Samples from the Moscow Region ranged from 10.35% to 11.48%, with an average of 10.87%. Based on these results and statistical analysis (confidence level P = 0.95), the moisture content standard for raw materials was set at no more than 12%.

Determination of total ash and ash insoluble in a 10% hydrochloric acid solution. Ash analysis was conducted in accordance with the requirements of the State Pharmacopoeia of the Russian Federation, 14th edition [5]. The results are presented in Tables 2 and 3.

Table 2 – Total ash content in the Chamaenerion angustifolium raw material

Region of raw material collection	f	Total ash, % (x)	$\bar{x}$	S	t_P (f)	Δx, %	E, %
Stavropol Krai	5	5.76; 6.13; 5.92; 5.64; 6.32; 5.85	5.94	0.2495	2.57	0.2618	4.41
Moscow region	5	5.84; 6.43; 6.31; 5.96; 6.04; 6.17	6.13	0.2213	2.57	0.2322	3.79

Table 3 – Content of ash insoluble in 10% HCl in the raw material of Stachys laxa

Region of raw material collection	f	Ash insoluble in 10% hydrochloric acid solution, % (x)	$\bar{x}$	S	t_P (f)	Δx, %	E, %
Stavropol Krai	5	1.39; 1.43; 1.52; 1.44; 1.48; 1.41	1.45	0.0476	2.57	0.0500	3.46
Moscow region	5	1.58; 1.64; 1.53; 1.66; 1.57; 1.52	1.58	0.0568	2.57	0.0596	3.76

Experimental data showed that the total ash content in the raw materials ranged from 5.64% to 6.43%, while the content of ash insoluble in a 10% hydrochloric acid solution ranged from 1.39% to 1.66%. Based on these data, and taking into account pharmacopoeial requirements for similar types of medicinal plant materials, the following standards were established: the total ash content should not exceed 7%, and the content of ash insoluble in a 10% hydrochloric acid solution should not exceed 2%.

Determination of impurity content, degree of grinding, and extractive substances. The impurity content (organic and mineral), the degree of grinding of raw materials, and the content of extractive substances extracted with 70% ethyl alcohol were assessed in accordance with the instructions of the State Pharmacopoeia of the Russian Federation, 14th edition [5,6,7]. The results are presented in Table 4.

Table 4 – Indicators of impurity content, degree of fragmentation, and extractive substances in the raw material of «Chereda drooping herb"

Indicator (x), %	Growing region	
	Stavropol Krai	Moscow region
Organic impurity, no more than	1.54 ± 0.06	1.41 ± 0.07
Mineral impurity, no more than	0.69 ± 0.02	0.53 ± 0.03
Extractive substances extracted with 70% ethyl alcohol	38.56 ± 1.74	32.87 ± 1.40
Whole raw material. Particles passing through a 3 mm sieve.	3.51 ± 0.16	3.18 ± 0.17
Crushed raw materials. Particles that do not pass through a 7 mm sieve.	4.17 ± 0.19	3.46 ± 0.16
Crushed raw material. Particles passing through a sieve with 0.18 mm holes	3.68 ± 0.17	3.55 ± 0.19

According to the table data, the content of organic impurities in the raw materials ranged on average from 1.41% to 1.54%, mineral impurities – from 0.53% to 0.69%. The content of extractive substances extracted by 70% ethyl alcohol varied within the range from 32.87% to 38.56%. Analysis of the degree of grinding showed that for whole raw materials, the proportion of particles passing through a sieve with 3 mm holes did not exceed 3.51%, and for crushed raw materials, the proportion of particles not passing through a 7 mm sieve was up to 4.17%, and the proportion of particles passing through a 0.18 mm sieve was up to 3.68%.

Based on these results and generally accepted pharmacopoeial norms, the following quality standards were established:

– organic impurity content – no more than 2%.

– mineral impurity content – no more than 1%.

– the content of extractive substances extracted with 70% ethyl alcohol is not less than 30%.

– for whole raw materials: particles passing through a sieve with 3 mm holes – no more than 5%.

– for crushed raw materials: particles that do not pass through a sieve with 7 mm holes – no more than 5%; particles that pass through a sieve with 0.18 mm holes – no more than 5%.

Development of methods for the qualitative determination of the main groups of biologically active compounds

Chromatographic determination of polyacetylene compounds. Thin-layer chromatography was used to determine polyacetylenes. The following method was developed as a result of the research: "1 µl of a hexane solution of essential oil extracted from Bidens sylvatica herb and a hexane solution of a standard phenylheptatriene sample are applied to the starting point of a chromatographic plate. After removing the solvent, the plate is placed in a chromatographic chamber pre-saturated with mobile phase vapor (hexane). Chromatography is carried out in an ascending manner until the solvent front has traveled approximately 90% of the plate length. Once complete, the plate is removed and dried."

For visualization, the dried plate is uniformly sprayed with a vanillin solution in concentrated sulfuric acid and heated at 100–105°C. This should result in the formation of at least two distinct adsorption zones. Identification of the polyacetylene compound zones is based on two criteria: when irradiated with UV light at 365 nm, the zone exhibits bright yellow fluorescence and has an Rf value in the range of 0.5–0.55.

Chromatographic determination of flavonoid compounds. Preparation of solutions. Rutin solution : approximately 0.005 g of rutin (rutin trihydrate) is dissolved in 10 ml of 95% ethyl alcohol. Shelf life: no more than 3 months.

Quercetin SO solution: approximately 0.005 g of quercetin dihydrate or anhydrous quercetin is dissolved in 10 ml of 95% ethyl alcohol. Shelf life is no more than 3 months.

1% diphenylboryloxyethylamine solution in 95% alcohol: dissolve 1.0 g of reagent in 100 ml of 95% ethyl alcohol. Shelf life: no more than 3 months.

5% polyethylene glycol (PEG) solution in 95% alcohol: 5.0 ml of PEG 400 is mixed with 100 ml of 95% ethyl alcohol. Shelf life: up to 6 months.

Sample preparation and chromatography. Approximately 1.0 g of crushed raw material (particles pass through a 0.5 mm sieve) is placed in a flask, 10 ml of 95% ethyl alcohol is added, a reflux condenser is attached, and the mixture is heated in a water bath for 10 minutes. The cooled extract is filtered. The resulting filtrate is the test solution.

A 30 µl sample of the test solution is applied to the starting line of a silica gel chromatography plate as a strip (10 x 3 mm). 5 µl of each rutin and quercetin RS solutions are applied simultaneously. The plate is air-dried.

The plate is placed in a chamber pre-saturated with mobile phase vapor (ethyl acetate – anhydrous formic acid – water, 40:4:6) for at least 30 minutes. Chromatography is performed in an ascending fashion until the front has traveled 80–90% of the plate's length. The plate is removed and dried.

Identification and evaluation of results. The dried plate is heated for 2-3 minutes at 100–105°C. The warm plate is sequentially treated with a 1% solution of diphenylboryloxyethylamine and a 5% solution of PEG, then kept at room temperature for 30 minutes.

When viewed in daylight, the chromatogram of the sample should show two zones: the rutin zone (yellow, yellow-orange, or orange) and the quercetin zone located above (yellow, yellow-orange, or orange). The chromatogram of the test solution should show a distinct yellow zone located above the obligatory red or violet-red zone.

To confirm the results, the chromatogram is viewed under UV light at a wavelength of 365 nm.

Conclusion. The comprehensive research conducted allowed us to develop a quality indicator system for the medicinal plant material "Sequina drooping grass." During the study, diagnostically significant external and microscopic features were identified, enabling reliable identification of the raw material. Clear descriptions of the morphological characteristics were proposed for both whole and crushed raw materials.

Based on experimental data obtained for samples from various growing regions and taking into account the requirements of the State Pharmacopoeia of the Russian Federation, the following standardized numerical indicators were established:

- humidity – no more than 12%;
- total ash – no more than 7%;
- ash insoluble in 10% hydrochloric acid solution – no more than 2%;
- organic impurities – no more than 2%;
- mineral impurities – no more than 1%;
- extractive substances extracted with 70% ethyl alcohol – not less than 30%;
- degree of grinding: for whole raw materials – particles passing through a sieve with 3 mm holes, no more than 5%; for crushed raw materials – particles not passing through a 7 mm sieve, and particles passing through a 0.18 mm sieve, no more than 5%.

Methods for the qualitative chromatographic (TLC) determination of polyacetylene and flavonoid compounds, which are necessary for confirming the authenticity of raw materials, have been developed and tested.

The data obtained opens up prospects for the development of various biologically active supplements (BAS) based on the raw material "Bed-Quill Grass." Due to its flavonoid and polyacetylene compounds, the raw material can serve as the basis for the development of:

- Dietary supplement with antioxidant and capillary-strengthening activity;
- means that promote the normalization of metabolic processes;
- anti-inflammatory and dermatotropic complex drugs.

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Сбор данных, проведение экспериментальных исследований. Подготовка и редактирование текста – составление черновика рукописи.

Дмитрий Алексеевич Коновалов

Проведение исследования – интерпретация и анализ полученных данных. Утверждение окончательного варианта – принятие ответственности за все аспекты работы, целостность всех частей статьи и её окончательный вариант.

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Contribution of the authors:

Aida M. Nasukhova

Collected data from published sources, carrying out experimental research. Prepared and edited the text – drafted the manuscript.

Dmitry A. Konovalov

Conducted the study – interpreted and analyzed the data.Approved the final version – accepts responsibility for all aspects of the work, the integrity of all parts of the article, and its final version.