Phytochemical study of *Glycyrrhiza pallidiflora* Maxim.

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Abstract. This article presents the results of the primary phytochemical study of Glycyrrhiza pallidiflora Maxim. introduced in the southeast of Western Siberia. The qualitative and quantitative composition of several primary and secondary metabolites in underground and aboveground organs of the plant were studied in different years of introduction and vegetation period. The presence of triterpene saponins, coumarins, chromones, chalcones, aurones, 5-oxy-flavones, 5-oxy-flavonols, flavan-3-ols, and proanthocyanidins was determined. The total content of phenolic compounds in the grass reached 2.83±0.22% and in the roots up to 0.77±0.27%. Eighteen proteinogenic amino acids were identified, including 8 essential and 10 nonessential amino acids. The seeds were found to accumulate 4.27±0.25% of fatty oils with a predominance of triglycerides of polyunsaturated acids.

1 Introduction

Licorice (Glycyrrhiza pallidiflora Maxim.), a member of the legume family, was included in the collection of the “Apothecary Garden” of the Kuzbass Botanical Garden in 2016. Since then, the study of the introduction and phytochemistry of this plant has been initiated [5].

Licorice is endemic to the Far East, occurring in the territory of the Middle Amur region and Lake Khanka. It grows on sandy-pebble and clay soils [1, 3–6]. Its roots are used in traditional medicine as an enveloping and expectorant agent and mild laxative [1].

Recently, there has been a growing interest in the raw materials of this plant as a source of biologically active compounds (BACs) for use in agriculture and medicine. There is scientific evidence for its use as a feed additive. Flour obtained from dried rhizomes is used to prevent infertility in cows, which can be explained by the accumulation of isoflavones (phytoestrogens) [2, 3–5].

The results of the introduction studies carried out in 2016–2023 showed the high resistance of the species to the conditions of the forest-steppe natural climatic zone of the southeast of Western Siberia [3–6].

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This work summarizes the primary results of the phytochemical study of the raw materials of Glycyrrhiza pallidiflora Maxim. introduced in the “Apothecary Garden” of the Kuzbass Botanical Garden.

2 Materials and methods

The materials used in the study included roots, grass, and seeds of pale-flowered licorice, collected in various years of introduction and vegetation periods. The grass was harvested during the flowering period, while the underground organs were collected during the fruiting period in 2016–2023.

Phytochemical studies were carried out according to conventional methods and current OFS and GOST standards [3–6]. To carry out the analysis, extracts were prepared in a ratio of 1:10 using various extractants: for flavonoids – 70% ethanol, for coumarins – 95 % ethanol, and for saponins – 50% ethanol and water [4].

Statistical treatment of the obtained data was carried out using Statistica 6.1 and Microsoft Office Excel 2007 software packages.

3 Results and discussion

Qualitative analysis of alcoholic and aqueous extracts of raw materials of pale-flowered licorice confirmed the presence of the following BACs: triterpene saponins, coumarins, chromones, flavonoids, chalcones and aurones with a free ortho-hydroxyl group in the B-ring, 5-oxy-flavones, and 5-oxy-flavonols. The presence of flavan-3-ol derivatives and condensed tannins (proanthocyanidins (PACs)) in the grass was determined for the first time [3].

The dynamics of accumulation of secondary phenolic metabolites in the aboveground part of the plant in different years of introduction showed that the largest amount of phenolic compounds is found in the grass during flowering period, regardless of age. The total content of phenolic compounds relative to gallic acid, determined by the Folin-Ciocalteu method, varied from 2.07±0.12% to 2.83±0.22% [3]. The underground part of the plants was characterized by a lower concentration of phenolic compounds in all the years of the study, varying from 0.51±0.34% in the samples of the third year to 0.77±0.27% in the fourth year of introduction [3]. No correlation was found between the amount of phenolic compounds in the grass and the root system or the age of the plants. The highest concetration of phenolic compounds (2.83±0.22%) was observed in the fourth year of grass introduction.

In order to determine the quantitative content of flavonoids in pale-flowered licorice grass, a differential spectrophotometry technique was developed based on the complexation reaction of rutin with aluminum (III) chloride, with the measurements being carried out at a wavelength of 408 nm [4]. It was found that the maximum extraction of flavonoids from raw materials with a particle size of 0.5 mm was achieved by 70% ethyl alcohol solution following heat treatment in a water bath for 60 minutes. The content of flavonoids in the grass collected during the flowering period of the plant increased from 1.26±0.04% to 2.44±0.03% in relation to the age of the plant. The maximum accumulation of flavonoids in the grass was observed in the third year of introduction. The increase in the amount of flavonoids up to the third year of introduction probably indicates the adaptation of the plants to new conditions, in particular, to insolation and increased nutrient availability compared to the soil conditions of the natural habitat.

Phytochemical analysis showed that the accumulation of PACs in pale-flowered licorice occurs only in the aboveground part of the plant. The study of the products of acid-catalyzed depolymerization of the total amount of PACs isolated from the aqueous alcohol extraction of licorice herb by column chromatography on a polyvinyl sorbent showed that their polymer structure is based on monomers of catechin or epicatechin [5]. By using the modified Porter method, based on the acid depolymerization of PACs to cyanidin chloride, it was found that their accumulation in grass plants increases from 1.46±0.06% to 2.61±0.11% depending on the age of the plant [3]. The annual increase in PAC content is 20–30%.

For the study of amino acid composition, licorice raw materials obtained in the third and fourth years of introduction (2018 and 2019) were used, while for the indicators of feed value, materials from the first and fourth years (2016 and 2019) were evaluated. The research was carried out in accordance with the requirements of the current GOST standards in cooperation with the accredited laboratory of biochemistry SibRDTIAH SFSCA RAS, Novosibirsk.

8 essential (valine, leucine, isoleucine, methionine, phenylalanine, tryptophan, threonine, and lysine) and 10 nonessential proteinogenic amino acids (alanine, proline, glycine, cysteine, tyrosine, serine, glutamic and aspartic acids, arginine, and histidine) were identified in the of pale-flowered licorice grass collected during flowering [6].

The predominance in the content of amino acids with polar (hydrophilic) negatively charged radicals (acidic) was established – 2.105 and 4.862% in the third and fourth years of introduction, respectively. Amino acids with nonpolar (hydrophobic) radicals (neutral) exhibited a slightly lower content (2.185–3.610%). The lowest specific gravity was established for amino acids with polar positively charged radicals (basic) (0.645–1.684%). The ratio of essential (essential) and nonessential amino acids is approximately 1:3, indicating a predominance of nonessential amino acids [6].

The analysis of feed value indicators revealed that the content of crude fat, crude protein, crude ash, Barnstein protein, nitrogen-free extractives (NFE), and phosphorus reached the highest values in the third and fourth years of introduction. The metabolic energy (ME) and the energetic feed unit for raw nutrients in the pale-flowered licorice grass averaged 8.98 MJ/kg (with annual variation of 8.31–9.76 MJ/kg) and 0.90 (annual variation of 0.83–0.98) for cattle and 9.34 MJ/kg (annual variation of 8.56–10.915) and 0.94 (annual variation of 0.86–1.02) for sheep, respectively, over four years, reaching the highest values in the third and fourth year of introduction. Dried licorice grass surpasses legume hay in terms of crude protein content by 97.9%, crude fat by 64.0%, NFE by 13.3%, and ME by 34.0%.

In the conditions of the forest-steppe natural climatic zone of the Kemerovo region (Kuzbass), the seeds of the pale-flowered licorice ripen completely in a short vegetation period from May to August. The fatty oil content in seeds of licorice was 4.27 ± 0.25% obtained by hexane extraction. An opaque, sedentary, oily orange liquid with a particular odor was obtained. The fatty oil is practically insoluble in water, slightly soluble in alcohol, and easily soluble in chloroform, petroleum ether, and hexane. The oil shows significant refraction with a refractive index of 1.4800 at 20ºC. This value is typical for oils with a high content of triglycerides of linoleic and linolenic acid. In addition, a high iodine number (146.03±0.92) suggests the predominance of polyunsaturated fatty acids in the composition of triglycerides. The saponification number is 215. Thin layer chromatography (TLC) method showed the presence of carotenoids and tocopherols, which are natural antioxidants of fatty oils.

Conclusion

In the raw material of *Glycyrrhiza pallidiflora*, the presence of triterpene saponins, coumarins, chromones, flavonoids, flavones, chalcones and aurones with a free ortho-hydroxyl group in the B-ring, 5-oxy-flavones, and 5-oxy-flavonols was determined. For the first time, the presence of monomeric flavan-3-ols and condensed tannins – proanthocyanidins – was revealed in the pale-flowered licorice grass. The highest content of phenolic compounds 2.83±0.22% was observed in the 4th year of grass introduction.

A method developed for the quantitative determination of the amount of flavonoids in grass is based on the reaction of rutin complexation (the relative error of the method is ± 3.21%).

The amount of flavonoids in the raw materials harvested during the flowering period (1.22–2.44%) exceeded the content harvested during the fruiting period (0.98–1.65%), with the annual excess ranging from 13.1 to 41.0%. No regularities in the amount of flavonoids depending on the year of introduction were observed, which indicates that weather conditions may be the determining factor.

The predominance of amino acids with polar negatively charged radicals (acidic) was revealed in the grass (2.105 and 4.862% annually), and amino acids with non-polar radicals (neutral) were slightly inferior (2.185–3.610%). Amino acids with polar positively charged radicals (basic) were characterized by the lowest specific gravity (0.645–1.684%).

Feed value indicators for cattle and sheep reached the highest values in the third and fourth years of introduction. The dried licorice grass surpasses legume hay in terms of crude protein content by 97.9%, crude fat by 64.0%, NFE by 13.3%, and ME by 34.0%.

The fatty oil content of the seeds was 4.27±0.25%. The high refractive index of 1.4800 and an iodine number of 146.03± 0.92 indicate a significant content of unsaturated fatty acids. The presence of β-carotene and tocopherols was determined by thin-layer chromatography.

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