UDC 615.322:582.998.1
https://www.doi.org/10.34907/JPQAI.2021.99.12.004

STUDY OF NITROGEN-CONTAINING COMPOUNDS OF PRICKLY LETTUCE (*LACTUCA SERRIOLA* L.)

T.V. Korablyova, *Postgraduate Student of the Department of Pharmacognosy and Botany, Kursk State Medical University, Kursk, Russia, bubenhikova.ksmu@yandex.ru*
R.A. Bubenchikov, *Doctor of Pharmacy, Deputy Head of the Department for Standardization of Biological Medicines, the Department of Standardization and Implementation of Scientific Development Management, "Scientific and Production Association for Medical and Immunobiological Drugs "Microgen", Moscow, Russia*

The object of the study was the air-dry herb of prickly lettuce (Lactuca serriola L.), harvested in 2019 in the Medvensky district of the Kursk region during the plant flowering period. The qualitative and quantitative composition of nitrogen-containing compounds of prickly lettuce herb was studied. The presence of nitrogenous bases in the herb was determined in water extracts using qualitative reactions and paper chromatography. The paper chromatography method revealed 6 compounds classified as nitrogenous bases with Rf 0.09, Rf 0.25, Rf 0.35, Rf 0.47, Rf 0.73, Rf 0.98. For the quantitative determination of nitrogenous bases, the method of G.A. Lukovnikov and A.I. Esyutina was used. This method is based on the determination of the optical density of nitrogenous bases with Reinecke salt. The content of the sum of nitrogenous bases in the prickly lettuce herb is 0.13±0.002%, including choline – 0.05±0.003%. The amino acid composition of lettuce herb is represented by 16 compounds: aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine; 8 of which are essential.

Keywords: Prickly lettuce (*Lactuca serriola* L.) herb, nitrogenous bases, amino acids, chromatography, photoelectric colorimetry, high performance liquid chromatography

Prickly lettuce (*Lactuca serriola* L.) of the sunflower family *(Asteraceae)* has long been used in folk medicine in Russia as an antipyretic, anti-inflammatory, analgesic, antitumor, blood purifying agent [1,2]. When taking prickly lettuce, appetite improves, headaches and cough decrease; it can treat jaundice, leprosy, insomnia. Boiled lettuce is used to increase the secretion of milk and to treat the chest organs. Lettuce leaves are used in case of ligament tension in the form of bandages [2]. The prickly lettuce has attracted the attention of scientists since the discovery of milky juice in it, which is used for the same purpose as lettuce herb [2]. Experimental studies of extracts obtained by various extraction solvents from the aboveground part of lettuce have established the antioxidant activity by the reaction of free radicals with 1,1-diphenyl-2-picrylhydrazyl. The most pronounced activity was observed in the ethyl acetate fraction. The antioxidant effect was also found in flavonoids and sesquiterpene 11/3, 13-dihydrolactucin isolated from lettuce herb [3]. Methanol extract from the herb showed antispasmodic, analgesic, vasodilating, antitumor, and bronchodilatory effects [4–6]. Triterpene compounds isolated from the aboveground part of the plant exhibit anti-inflammatory, antibacterial, antitumor, cytostatic, and antimalarial

effects [5]. In the literature, there is evidence that lettuce exhibits an antidiabetic property, reducing blood sugar levels [2].

Prickly lettuce is represented by annual or biennial herbaceous plants growing on the territory of the European part of Russia, in the Far East, in Western and Eastern Siberia, in the Caucasus [1]. In the regions of central Russia, it grows on waste lands, weed-grown places, roadsides, in vegetable gardens, orchards [1]. It naturally forms significant thickets and has a sufficient raw material base. However, to use lettuce as a raw material source, data on its chemical composition is necessary. In the literature, information about the composition of biologically active substances of lettuce is rare, and the studies were conducted mainly by foreign scientists. The most common information is about the presence of sesquiterpene lactones, which are found in all the organs of the plant, in the aboveground part, the steroid and triterpene compounds are also established; flavonoids are also identified in it. The leaves additionally contain a complex of vitamins [6]. The seeds were tested for the content of alkaloids, sesquiterpenoids, triterpenoids, essential and fatty oils [2,6].

Purpose of this work is study of nitrogen-containing compounds in the prickly lettuce herb.

MATERIALS AND METHODS

The aboveground part of the prickly lettuce collected in the Medvensky district of the Kursk region in 2019 during the flowering phase was selected as the material for the study. The collected raw materials were dried in the air in a shaded place. For the analysis of nitrogen-containing compounds, an average sample was used, from which an analytical sample was taken and crushed to a particle size of 1 mm. The determination of nitrogen-containing compounds included the determination of nitrogenous bases and amino acids.

For the qualitative analysis of nitrogenous bases and amino acids from the analytical sample of raw materials (5.0 g), an aqueous extract was obtained by three-time extraction with purified water of 50 ml, each extraction was carried out for 1 hour. The resulting aqueous extracts were combined, evaporated to a volume of 25 ml under vacuum, and used for qualitative analysis of nitrogenous bases and amino acids. For the qualitative analysis of nitrogenous bases, qualitative reactions were used: with Mandelin reagent, with phosphoric-tungsten acid, with a 3% diamond green solution, and with hydrochloric acid; as well as the method of paper chromatography was used, the solvent system in which was the system "n. butanol – acetic acid – water" (4:1:2), the developer was iodine vapor [7].

Qualitative analysis of amino acids was performed using the ninhydrin reaction and the thin-layer chromatography method [8]. Chromatographic analysis was performed on Sorbfil plates using the solvent system "96% ethyl alcohol – concentrated ammonia" (16:4,5) and reliable samples of amino acids. The developer of the chromatogram was a 0.2% ninhydrin solution, after which the chromatogram was held in a drying cabinet at 100–105°C for a few minutes. At the same time, red-purple spots of amino acids appeared.

The quantitative analysis of nitrogenous bases was carried out by the photoelectrocolorimetric method, using the modified method of G.A. Lukovnikov and A.I. Esyutina. The method is based on the formation of colored complexes of nitrogenous bases contained in raw materials with Reineke salt, and the determination of their optical density. The extract was obtained with purified water and used to determine the choline and the amount of nitrogenous bases. To determine choline, hydrochloric acid was added to the resulting extract to provide pH=3 and a solution of Reineke salt, then placed in the refrigerator for 18 hours to obtain a precipitate of nitrogenous

bases. The precipitate of the resulting complex was separated by filtration, dissolved in acetone, and colorimetrated at a wavelength of 400±10 nm (blue light filter) on a photoelectrocolorimeter. Under the same conditions, a solution of a standard sample of choline with Reinecke salt was colorimetrated [7]. The amount of nitrogenous bases was determined in the same water extract, after adding a potassium permanganate 0.1 N solution to it and heating it in a water bath for 10 minutes to oxidize the nitrogenous bases to choline. Further determination was carried out according to the method of determining choline [7].

The amino acid composition was studied by high-performance liquid chromatography using an Agilent 1260 device with a fluorescent detector (FLD), for which aqueous extract was used. When determining the content of free amino acids, the aliquot of aqueous extract and standard solutions (in the form of aqueous solutions) of amino acids were derivatized with the reagent ACCQ FLUOR, mixed using a vortex mixer and incubated for 10 minutes at temperature of 55°C. The resulting solutions were introduced into a chromatographic column and analyzed using the following conditions: gradient elution method, mobile phase flow rate – 1 ml/min, excitation wavelength – 250 nm, emission – 395 nm [9].

RESULTS AND DISCUSSION

Positive results of qualitative reactions to the presence of nitrogenous bases indicate their presence in the prickly lettuce herb. By the method of thin-layer chromatography, 6 substances were found: with Rf 0.09, Rf 0.25, Rf 0.35, Rf 0.47, Rf 0.73, Rf 0.98, classified as nitrogenous bases. Photoelectrocolorimetric determination of nitrogenous bases showed that their sum is 0.13±0.002%, including choline 0.05±0.003%.

When conducting a qualitative analysis of amino acids, the formation of red-purple color

Table

AMINO ACID COMPOSITION

Name of amino acids	Content of amino acids	
	mg/100 g in raw material	% sum
Aliphatic amino acids *Monoaminomonocarboxylic*		
Alanine	4.89	10.53
Valine*	6.40	4.02
Leucine*	19.34	21.36
Isoleucine*	0.73	0.63
Glycine	12.24	9.39
Total content	43.61	45.93
Monoaminodicarboxylic		
Glutamine acid	9.29	3.55
Asparaginic acid	4.30	2.42
Total content	13.59	5.97
Diaminomonocarboxylic		
Arginine	20.52	16.95
Lysine*	1.63	3.08
Total content	22.15	20.03
Oxymonoaminocarboxylic		
Threonine *	3.60	1.94
Serine	10.79	4.55
Total content	14.39	6.49
Sulfur-containing substances		
Methionine*	0.34	7.50
Total content	0.34	7.50
Aromatic amino acids		
Tyrosine	3.16	0.60
Phenylalanine*	4.90	6.05
Total content	8.06	6.65
Heterocyclic amino acids		
Histidine*	2.64	4.36
Proline	14.79	3.07
Total content	17.43	7.43
Content of essential amino acids	39.58	

*Note: * – essential amino acids*

staining with ninhydrin reagent indicates their presence in the prickly lettuce herb. Chromatographic analysis of the amino acid composition with a certain degree of confidence showed the presence of arginine, alanine, leucine, methionine, and glycine.

The results of the study of the amino acid composition by high-performance liquid chromatography revealed the presence of 16 amino acids, including 8 essential ones (see Table.)

The amino acid profile of prickly lettuce herb includes aliphatic (monoaminocarboxylic, monoaminodicarboxylic, diaminomonocarboxylic, oxymonoaminocarboxylic, sulfur-containing), aromatic, and heterocyclic amino acids. Among them, aliphatic amino acids predominat; their content is 85.92% in the total of amino acids.

Among the aliphatic amino acids, monoaminocarboxylic acids predominate, the content of which is 45.93% in the total of acids, followed by diaminocarboxylic acids (20.03%). The lowest content among them was found in monoaminodicarboxylic acids (5.97% in total). The content of aromatic (6.65%) and heterocyclic (7.43%) is approximately at the same level. The content of essential amino acids was 39.58 mg/100 g. Thus, the prickly lettuce herb can be considered as an additional source of amino acids.

CONCLUSIONS

1. The qualitative composition and quantitative content of nitrogen-containing compounds (nitrogenous bases, amino acids) of prickly lettuce (*Lactuca serriola* L.) herb have been established. The content of the sum of nitrogenous bases in the prickly lettuce herb is 0.13±0.002%, including choline 0.05±0.003%.

2. The qualitative and quantitative amino acid composition of the prickly lettuce herb has been established: amino acids are represented by 16 compounds, of which 8 are essential. Among the identified groups of amino acids, the maximum content was found in monoaminodicarboxylic amino acids (45.93%).

3. The conducted studies allowed us to expand the composition of biologically active substances of prickly lettuce herb, which can contribute to their further use in medical practice as sources of these groups of natural compounds, as well as contribute to their further study for creation of drugs based on them.

REFERENCES

1. *Gubanov I.A., Kiseleva K.V., Novikov V.S., Tikhomirov V.N. Illustrated key to plants of Central Russia. Volume 3: Angiosperms (dicotyledonous: eleutheropetalous).). – M.: Association of Scientific Publications KMK, Internet Technology Study.*
2. *Karomatov I.D., Ramazonovna A.M. Lettuce – a promising medicinal plant // Biology and integrative medicine. Phytotherapy [Electronic resource]: electronic scientific journal – 2018. – No. 4. – p. 122–129.*
3. *Kim D.K. Antioxidative components from the aerial parts of Lactuca scariola L. // Archives of pharmacological research. – 2001. – Vol. 24, №5. – P. 20–23.*
4. *Elsharkawy E., Alshathly M. Anticancer activity of Lactuca serriola growing under dry desert condition of Northern Region of Saudi Arabica // Journal of Natural Science Research. – 2013. – Vol. 3, №2. – P. 5–16.*
5. *Janbaz K.H., Latif M.F., Sagib F., Imran I., Zia-Ul-Haq M., De Feo V. Pharmacological effects of Lactuca serriola L. in experimental model of gastrointestinal, respiratory and vascular ailments // Evidence-based complementary and alternative medicine. – 2013. – P. 1–9.*
6. *Mohammad A. Traditional use of Kahu (Lactuca scariola L.) – a review // Global J.Res. Med. Plants & Indigen. Med. – 2013. – Vol. 2, Issue 6. – P. 465–474.*

7. *Pozdnyakova T.A., Bubenchikov R.A. Study of nitrogen-containing compounds of bog crane // Questions of biological, medical and pharmaceutical chemistry. – 2015. – No. 11. – p. 37–39.*
8. *Bubenchikova V.N., Levchenko V.N. Amino acid and mineral composition of the rush skeletonweed herb // Modern problems of science and education [Electronic resource]: Electronic scientific journal – 2015. – No. 5 (61). – Available at: http://science-education.ru/128–21450, free.*
9. *Sechin E.N., Marakaev O.A., Gavrilov G.B. Amino acid composition of vegetative organs Dactylorhiza maculate (L.) Soo (Orchidaceae) // Chemistry of vegetable raw materials. – 2019. – No. 2. – p. 135-143.*