INTERNATIONAL MULTIDISCIPLINARY JOURNAL FOR RESEARCH & DEVELOPMENT
SJIF 2019: 5.222 2020: 5.552 2021: 5.637 2022:5.479 2023:6.563 2024: 7,805
eISSN :2394-6334 https://www.ijmrd.in/index.php/imjrd Volume 12, issue 06 (2025)

UDK 615.014

DETERMINATION OF PROTEIN AND AMINO ACID COMPOSITION OF THE DEVELOPED DRY EXTRACT "IMMUNASHIP"

[1] Zuparova Zulfiya Ahror kizi , [2] Khudayshukurova Aziza Arslanbek kizi

[3] Ismoilova Guzaloy Mukhutdinovna

[1] Tashkent Medical Academy, Tashkent

[2] Termez branch of the Tashkent Medical Academy , Termez

[3] Tashkent Pharmaceutical Institute, Tashkent

e-mail: zazulfiya@gmail.com tel (number) +998935206696

Abstract. Herbal preparations that enhance immunity, due to the presence of a variety of biologically active substances in them, gently affect the body and restore impaired functions of the immune response. For the prevention of immune system disorders, preparations based on purple echinacea (*Echinacea purpurea)* , as they have a positive effect on the body's immunity to infectious agents and reduce the manifestations of allergic diseases. This article defines the protein and amino acid composition of the dry extract "Immunaship". The protein in the dry extract " Immunaship " was determined by the Kjeldahl method for the quantitative content of nitrogen. The amino acid composition of the dry extract was studied and 20 free amino acids were identified, with a quantitative content of 8.14969 mg/g, 10 of which are essential, which indicates the biological value of the dry extract " Imunaship " based on purple echinacea herb and rose hips.

Key words: Protein, amino acid composition, dry extract, Immunaship, HPLC, Kjeldahl method, purple echinacea, rose hip, immunity.

Introduction. Understanding the role of drugs that enhance immunity in the biosystems of a living organism is the key to creating new classes of effective dosage forms. The use of environmentally friendly plant materials is based on the deep relationship of natural components with the human body [1,2,3]. The healing properties of the plant are due to the action of a complex of biologically active substances contained in the plant, their harmonious interaction and optimal ratio when acting on the human body. Research into herbal medicines with the aim of introducing them into medical practice, developing optimal technologies for obtaining dry extract and studying the biologically active substances in them that determine their pharmacological action is very relevant[4,5,6,7].

Herbal preparations that enhance immunity, due to the presence of a variety of biologically active substances in them, gently affect the body and restore impaired functions of the immune response. To prevent immune system disorders, preparations based on purple echinacea (*Echinacea purpurea)*, as they have a positive effect on the body's immunity to pathogens of infectious diseases and reduce the manifestations of allergic diseases,[8,9,10,11,12].

The effectiveness of purple echinacea can be enhanced by combining it with medicinal plants with antioxidant action, as well as by optimizing the dosage form [13,14,15,16,17].

Purpose of the study Determination of protein and amino acid composition of the developed dry extract " Immunoship " based on purple echinacea herb and rose hips.

Materials and methods The object of the study was the extract from the soup "Immuna ship" based on the herb Echinacea purpurea and rose hips.
Identification of amino acid derivatives in the dry extract was carried out by HPLC using an Agilent chromatograph. Technologies 1200 with DAD detector, with 75 x 4.6 mm column Discovery H S C $_{18}$. The mobile phase consists of solution (A): 0.14 M sodium acetate and 0.05% triethylamino, at a solution pH of 6.4; Solution (B) consists of acetonitrile. Chromatographic analysis was carried out in the following mode: I - 1-6% / 0-2.5 min; II - 6-30% / 2.51-40 min; III - 30-60% / 40.1-45 min; IV - 60-60% / 45.1-50 min; V - 60-0% / 50.1-55 min, flow rate 1.2 ml/min, at UV detector absorption of 269 nm , at room temperature.
Protein was determined by the Kjeldahl method.

Experimental part

In plants, amino acids are found in a free and protein-bound state, and during extraction, both free and bound amino acids pass into the extract. When determining free amino acids in a dry extract, protein-bound amino acids and peptides interfering with the analysis were precipitated by centrifugation. For this, an exact volume of 1 ml of 20% trichloroacetic acid was added to 1 ml of the sample being studied . The sediment was precipitated in centrifuge cups for 10 min at a rotation speed of 8000 rpm. 0.1 ml was collected from the supernatant liquid and dried in an air-lyophilic dryer. The hydrolyzate was evaporated, the dry residue was dissolved in a mixture consisting of triethylamine - acetonitrile - water in a ratio of (1:7:1) and dried. In order to neutralize the acids, this procedure was repeated twice. Phenylthiocarbamyl derivatives (PTC) of amino acids were obtained with a reaction with phenylthioisocyanate according to the Steven method. A ., Cohen Daviel . The obtained data on the composition and quantitative content of amino acids are given in Table 1, the chromatograms of the standard sample and dry extract are shown in Fig . 1 and Fig . 2, respectively.[18,19,20,21,22]

Table 1

Composition of free amino acids in the dry extract " Immunaship "

No.	Name of amino acids	Content, mg/g
1	Aspartic acid	0.083098
2	Glutamic acid	0.274905
3	Serene, Ser	0.458546
4	Glycine, Gly	0.121465
5	Asparagine, Asn	0.241324
6	Glutamine, Gln	0.816401
7	Cysteine, Cys	2.396721
8	Threonine, Thr *	0.930596
9	Arginine, Arg *	0.658713
10	Alanine, Ala	0.104805
11	Proline, Pro	0.910415
12	Tyrosine, Tyr	0.401187

INTERNATIONAL MULTIDISCIPLINARY JOURNAL FOR RESEARCH & DEVELOPMENT
SJIF 2019: 5.222 2020: 5.552 2021: 5.637 2022:5.479 2023:6.563 2024: 7,805
eISSN :2394-6334 https://www.ijmrd.in/index.php/imjrd Volume 12, issue 06 (2025)

13	Valin, Val *	0.212527
14	Methionine, Met *	0.105826
15	Histidine, His *	0.106568
16	Isoleucine, Ile *	0.040607
17	Leucine, Leu *	0.08202
18	Tryptophan, Trp *	0.079156
19	Phenylalanine, Phe *	0.058455
20	Lysine HCl , Lys *	0.066333
Total		**8.149669**
Total content of essential amino acids		2.34

The composition of the dry extract was studied and 20 free amino acids were identified, with a quantitative content of 8.14969 mg/g, 10 of which are essential, which indicates the biological value of the dry extract " Imunaship ".

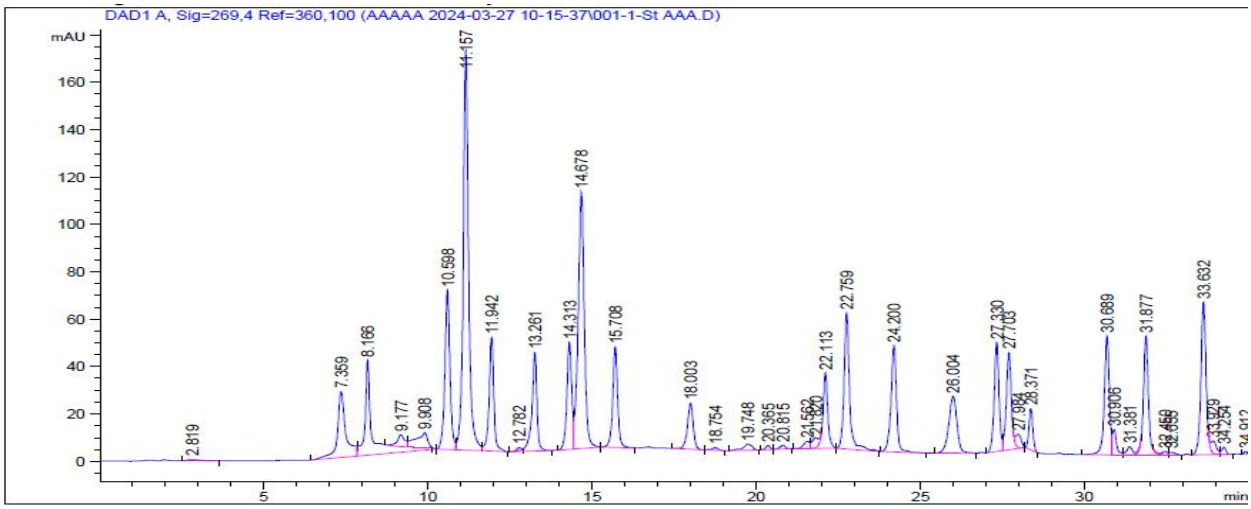

Fig. 1. Chromatogram of standard samples of free amino acids

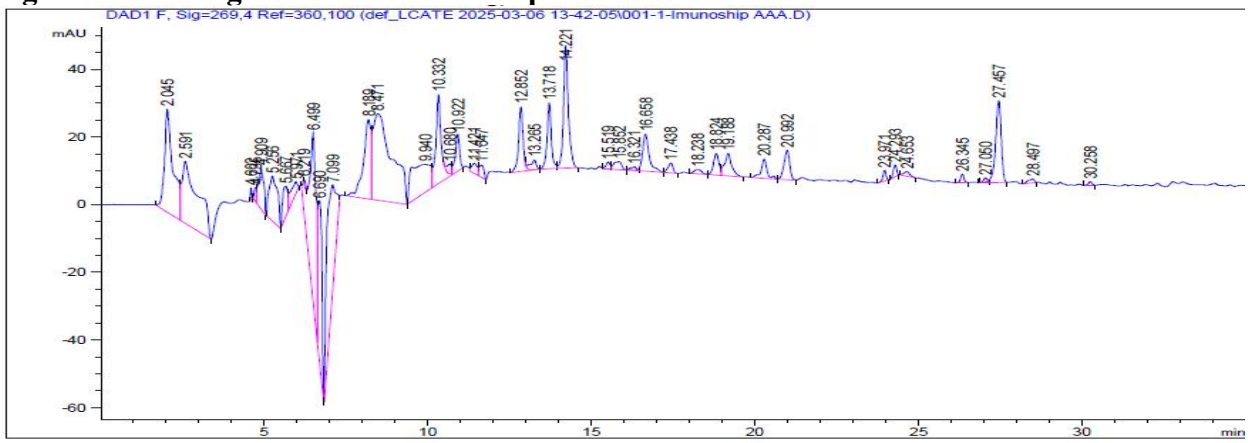

Fig. 2. Chromatogram of the amino acid composition of the dry extract "Imunaship"

Determination of the amount of total protein. The method consists of determining nitrogen according to Kjeldahl with subsequent recalculation into protein. The essence of the method consists of decomposing the organic substance of the sample with boiling concentrated sulfuric acid to form ammonium salts, converting ammonium into ammonia, distilling it into an acid solution, quantitatively accounting for ammonia using the titrimetric method and calculating the nitrogen content in the material being studied.[23,24,25]

From the averaged crushed homogeneous sample of the test specimen, an exact sample was weighed in a test tube for analysis, with an error of no more than 0.1%. The sample was quantitatively transferred to a Kjeldahl flask. Then the experiments were carried out according to the methodological instructions [1].

Processing of results: The mass fraction of nitrogen (X) in the test sample as a percentage of its mass during the distillation of ammonia into sulfuric acid was calculated using the formula

$$X = \frac{(V_1 - V_0) \times K \times 0{,}0014 \times 100}{M}$$

Vo – the volume of 0.1 mol/ l sodium hydroxide solution used for titration of 0.05 mol/l sulfuric acid in the control experiment, ml. *V1* – the volume of 0.1 mol/l sodium hydroxide solution used for titration of sulfuric acid in the test solution, ml; *TO* – correction to the titer of 0.1 mol/l sodium hydroxide solution;[24,25]

0.0014 – the amount of nitrogen equivalent to 1 ml of 0.05 mol/l sulfuric acid solution;

M – weight of sample, g. The final test result was the arithmetic mean of the results of five parallel tests. The results were calculated to the third decimal place and rounded to the second decimal place.

The mass fraction of nitrogen in terms of dry matter of the product (X3), in percent, was calculated using the formula:

$$X_3 = \frac{X_1 \times 100}{100 - W}$$

X 1 – mass fraction of nitrogen in the test sample, %; *W* – humidity of the test sample, %.

The mass fraction of protein (Y) in percent was calculated using the formula: $Y = K$ Ч X , where *TO* – nitrogen to protein conversion factor: with moderate lipid content – 6.38;

Conclusion The protein in the dry extract "Immunaship" was determined using the Kjeldahl method based on the quantitative nitrogen content. The amino acid composition of the dry extract was studied and 20 free amino acids were identified, with a quantitative content of 8.14969 mg/g, 10 of which are essential, which indicates the biological value of the dry extract "Imunaship".

Literature

1. Z.A. Zuparova, N.K. Olimov, G.M. Ismoilova, M.X. Tursunova «PRECLINICAL STUDIES OF DRY EXTRACT OF THE HERB OF ECHINACEA PURPUREA PRODUCED BY MEANS OF PREEXTRACTION» 湖南大学学报 (自然科学版) 48 (10)//2021

2. З.А. Зупарова, Г.М. Исмоилова «Сравнительный анализ содержания полисахаридов в эхинацеи пурпурной выращиваемой в различных регионах» Innovations in life sciences, 148-149//2023

3. S.A. Jabbarova, Z.A. Zuparova, G.M. Ismoilova «CHROMATOMASS-SPECTROMETRIC STUDY OF DRY EXTRACTS OF SEDUM L. OBTAINED BY

DIFFERENT SOLVENTS»/ Евразийский журнал медицинских и естественных наук 4 (5), 44-45//2024

4. ZA Zuparova, GM Ismoilova «Determination of Some Technological Properties and Authenticity of Immunacea Bio Tablets»/Best Journal of Innovation in Science, Research and Development 3 (1), 29-35//2024

5. X Kamilov, Z Abraeva, S Yusupova, G Djanaev «Development of composition and technology of antidiabetic tablets based on medicinal plants»/ BIO Web of Conferences 149, 01047//2024

6. З.А .Зупарова, Г.М. Исмоилова, В.Р. Хайдаров «ПОЛУЧЕНИЕ СУХОГО ЭКСТРАКТА ИЗ ЭХИНАЦЕИ ПУРПУРНОЙ МЕТОДОМ ПОЛИЭКСТРАКЦИИ И ИЗУЧЕНИЕ НЕКОТОРЫХ ФИЗИКО-ТЕХНОЛОГИЧЕСКИХ СВОЙСТВ ТТўқ қизил эхинацеядан полиэкстракция усули билан …»/O'ZBEKISTON FARMATSEVTIK XABARNOMASI, 23-24//2021

7. Z.A.. Zuparova, N.K.. Olimov, G.M. Ismoilova, B.J. Khasanova «Determination of high quality of echinaceae purpurae herba grown in Uzbekistan and the prospect of creating immunomodulatory medicinal products on its base»/International Journal of Psychosocial Rehabilitation 24 (4), 2355-2366//2020

8. Z.A. Zuparova, G.M. Ismoilova «Isolation and Study of Dry Extract from Echinacea Purpurea»/Global Journal of Medical Research: B Pharma, Drug Discovery, Toxicology//2022

9. Худойшукурова А.А., Зупарова З.А. ОПРЕДЕЛЕНИЕ КОЛИЧЕСТВА ГОРОКСИКОРИЧНЫХ КИСЛОТ В ПРЕПАРАТЕ «ИММУНАШИП» //Редакційна колегія. – 2024. – С. 122.

10. Зупарова З. А., Ризаев К. С. АССОРТИМЕНТНЫЙ АНАЛИЗ ИММУНОМОДУЛИРУЮЩИХ ЛЕКАРСТВЕННЫХ СРЕДСТВ //ЗАРЕГИСТРИРОВАННЫХ В РЕСПУБЛИКЕ УЗБЕКИСТАН. – №. 2022.

11. Kamilov X. et al. Development of composition and technology of antidiabetic tablets based on medicinal plants //BIO Web of Conferences. – EDP Sciences, 2024. – Т. 149. – С. 01047.

12. Зупарова З. А. ТЕХНОЛОГИЯ ПОЛУЧЕНИЯ ТАБЛЕТОК «ИММУНАЦЕЯ БИО» НА ОСНОВЕ ВЫСУШЕННОГО СОКА ЭХИНАЦЕИ ПУРПУРНОЙ //FARMATSIYA. – С. 80.

13. Зупарова З. А. и др. Изучение ассортимента иммуномодулирующих и иммуностимулирующих лекарственных средств в 2016-2021 гг., зарегистрированных в республике Узбекистан //Ремедиум. Журнал о российском рынке лекарств и медицинской технике. – 2021. – №. 4. – С. 84-87.

14. G.I.Sadikova, Z.A.Zuparova, G.I.Ismoilova, M.A.Mamadjanova « ИЗУЧЕНИЕ ТЕХНОЛОГИЧЕСКИХ СВОЙСТВ И ОПРЕДЕЛЕНИЕ ФЛАВОНОИДНОГО СОСТАВА СУХОГО ЭКСТРАКТА ПИОНА ЛЕКАРСТВЕННОГО»/ Farmatsiya va farmakologiya/Farmatsevtika ta'lim va tadqiqot instituti//2024/№4(10)

15. G.I.Sadikova, Z.A.Zuparova, G.I.Ismoilova «ОПРЕДЕЛЕНИЕ АМИНОКИСЛОТНОГО СОСТАВА РАЗРАБОТАННОГО СУХОГО ЭКСТРАКТА НА ОСНОВЕ ПИОНА ЛЕКАРСТВЕННОГО»/ Farmatsevtika jurnali/Toshkent farmatsevtika inatituti//2025/Tom34, №1