American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

Journal Website: https://theusajournals.com/index.php/ajbspi

Research Article

"HYPOSEDAF" DRY EXTRACT'S AMINO ACID AND ELEMENTAL COMPOSITION STUDY

Submission Date: January 21, 2024, Accepted Date: January 26, 2024,
Published Date: January 31, 2024
Crossref doi: https://doi.org/10.37547/ajbspi/Volume04Issue01-16

Safarova Diyora Tolibovna
Assistant Of The Tashkent Pharmaceutical Institute, Uzbekistan

Maksudova Firuza Khurshidovna
Tashkent Pharmaceutical Institute, Doctor Of Pharmaceutical Sciences, Associate Professor, Uzbekistan

Karaeva Nargizakhan Yuldash Qizi
Assistant Of The Tashkent Pharmaceutical Institute, Uzbekistan

ABSTRACT

Research was undertaken to examine the amino acid and elemental composition of "Hyposedaf", a dry extract known for its hypotensive properties. Quantitative analyses of both micro and macro elements were conducted using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Additionally, the amino acid content was quantitatively analyzed through High-Performance Liquid Chromatography (HPLC). This study identified 20 different amino acids in the "Hyposedaf" dry extract, 10 of which are essential. Notable amino acids, such as histidine, cysteine, tyrosine, methionine, valine, asparagine, and leucine, amounted to a total of 29.15 mg. Of all detected amino acids, 46.5% were classified as non-essential, while 53.5% were essential. The elemental analysis of the "Hyposedaf" dry extract revealed 61 different elements, including 7 essential elements (Co, Cr, Cu, Fe, Mn, Se, Zn) and 4 conditionally necessary elements. Key elements like potassium, calcium, sodium, zinc, iron, magnesium, and phosphorus, found in substantial amounts, are crucial for human health. The concentrations of heavy metals and arsenic in the dry extract were within safe limits according to the Sanitary-epidemiological rules and norms, complying with the quantitative standards State Pharmacopoeia of the Republic of Uzbekistan (Volume 1, Issue 1) and State Pharmacopoeia of the Russian Federation XIV (RF) [1,2,3]. The findings indicate that "Hyposedaf" dry extract possesses significant value, offering potential for medical application and the development of other medicinal forms.

KEYWORDS

Dry extract, micro and macro elements, amino acids, HPLC method, mass spectrometry.

INTRODUCTION

Extensive research has established the critical role of minerals and amino acids in the human body, acting as fundamental nutrients for synthesizing all proteins. Besides contributing to brain function, including intellectual activity, motivation, and general mental state, amino acids play a vital role in maintaining overall well-being. These nutrients are key components of all human tissues, such as muscles. Amino acids and chemical elements not only exhibit specific pharmacological effects but also demonstrate synergism with various substances, paving the way for the development of combined drugs with multiple functional properties derived from plants. Importantly, macro and micronutrients, as well as amino acids sourced from plants, are more effectively absorbed by the human body, owing to their "biological" quantities present in plants. Furthermore, amino acids assist in the optimal performance of mineral substances within the human body. The study of amino acid and mineral compositions in medicinal plants and phytopreparations is vital for the creation of new pharmaceutical drugs [4].

Currently, it's essential to understand the trace element content in the entire body of medicinal plants, including their separate generative and vegetative organs - roots, stems, leaves, buds, flowers, fruits, and seeds. This knowledge significantly expands the scope of their utilization. In plants, bioelements are found in forms that are absorbable by the human body, such as amino acids, vitamins, proteins, etc., and they are present in complex forms. This fact underscores the importance of medicinal plants as sources of biologically active substances and elements. The investigation of the elemental composition of medicinal plants and their based preparations is not only necessary for determining their value but also for preventing toxic and carcinogenic properties due to increased levels of heavy metals and arsenic [5,6].

Considering the above, the feasibility of using medicinal plant raw materials or extracts based on them in healthcare practice involves studying their amino acid and elemental composition. Amino acids are crucial for plant development, occurring during photosynthesis and participating in a wide range of biochemical reactions that support optimal growth and development [7,8,9].

Object of the Study: The aim of this study is to investigate the amino acid and elemental composition of the dry extract of "Hyposedaf".

Experimental Part: The research focused on the dry extract of "Hyposedaf", conditionally named and derived from various plant raw materials, including peppermint leaves (Folia Menthae piperitae L.), Turkistan lion's tail grass (Herba Leonuri turkistanicae), dark-red hawthorn fruits (Fructus Crataegi sanguineae Pall), and field horsetail grass (Herba Equiseti arvensis). This extract complied with the specifications outlined in the publications State Pharmacopoeia of the Russian Federation and XIV State Pharmacopoeia (RF) [10,11].

In the first phase of our research, we concentrated on the elemental composition of "Hyposedaf" analyzing its macro and microelements through Inductively Coupled Plasma Mass Spectrometry (ICP MS) as per the methodological guidelines [12].

In the second phase, the amino acid composition of the "Hyposedaf" dry extract was examined. This involved isolating free amino acids and conducting their quantitative analysis by comparing the retention times and peak areas of a standard sample with those of amino acids obtained from phenylthiocarbamyl.

Results and Discussion: The "Hyposedaf" dry extract was analyzed using the highly sensitive Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method. This technique enabled the simultaneous determination of numerous elements in a single sample with very low detection limits. The mass-spectral analysis was developed in Russia and validated in Uzbekistan (MBI) UzO`U 0677:2015 (MBI №499-AEM/MS), facilitating the determination of 61 elements (Na, Mg, Al, P, K, Ca, Ti, Mn, Fe, Li, Be, B, Sc, V, Cr, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Pt, Au, Tl, Pb, Bi, Th, U).

This method utilized argon inductively coupled plasma as an ion source. The test sample, weighing 0.1 g, was introduced into the plasma in solution form. Prior to measurement, the sample underwent solubilization. The resulting solution, containing the test sample, was aerosolized with a stream of argon and introduced into the plasma. The core temperature of the plasma reached up to 9000°C, at which the sample was desiccated, and the molecular elements transitioned to an atomic state, becoming ionized to form positive ions. These positive ions, along with electrons and neutral particles, were channeled through cone-shaped interfaces into the vacuum segment of the analyzer, passing through several electric ion lenses that filtered out electrons and neutral particles from the ions.

The test sample and a standard sample, each weighing 0.1 g, were placed in Teflon bags. Decomposition of the test, standard, and control samples was performed in a system involving Teflon bags with lids (Hot Block, Environmental Express). The Hot Block, consisting of 36 or 40 cells depending on the model, allowed for the heating of dissected samples in large volumes. This decomposition process utilized a mixture of HF, $HClO_4$, and HNO_3 acids in an open system to fully dissolve the test sample. After removing the bags from the heating

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

plate and allowing them to cool, 3 ml of concentrated hydrochloric acid was added to each. The resultant solutions were transferred to polyethylene tubes, diluted with distilled water, mixed, and subsequently analyzed. Elemental measurements in aqueous solutions were conducted within a mass range of 5 to 240. The analysis of each sample, ranging from 36 to 40 samples, took approximately 2-3 minutes. Following each measurement, the spray system was thoroughly rinsed with a 2% nitric acid solution and water for 0.5-1 minute.

The analysis of the elemental composition of the "Hyposedaf" dry extract is presented in Table 1.

Table 1.

The elemental composition of the "Hyposedaf" dry extract

Element	Quantity, mkg/g	Element	Quantity, mkg/g
Makroelementlar		Gallic, Ga	0,170
Potassium, K	65 000	Yttrium, Y	0,150
Calcium, Ca	10 000	Neodymium, Nd	0,130
Magnesium, Mg	9 100	Antimony, Sb	0,110
Millielements		Tin, Sn	0,076
		Tory, Th	0,071
Phosphorus, P	4000	Zirconium, Zr	0,067
Sodium, Na	1300	Platinum, Pt	<0,05
Aluminum, Al	460	Gold, Au	<0,05
Iron, Fe	280	Praseodym, Pr	0,04
Manganese, Mn	53,0	Samari, Sm	0,028
Zinc, Zn	47,0	**Nanoelements**	
Strontium, Sr	47,0	Gadolinium, Gd	0,018
Rubidium, Rb	39,0	Tantalum, Ta	<0,01
Boron, B	32,0	Thallium, Tl	<0,01
Microelements		European, Eu	0,01
Titan, Ti	13,0	Dysprosium, Dy	0,01
Copper, Cu	6,90	Ermiy, Er	0,01
Nickel, Ni	4,10	Ittermiy, Yb	0,01
Lithium, Li	2,10	Terbiy, Tb	<0,01
Molybdenum, Mo	1,40	Tuly, Tm	<0,01
Chrom, Cr	1,20	Gafni, Hf	<0,01
Silver, Ag	1,10	Berelli, Be	0,006
Selenium, Se	0,50	Golmi, Ho	0,006
Cerium, Ce	0,320	Indium, In	0,001

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

The subsequent section details the quantitative composition of elements in the dry extract, measured in micrograms per gram (as classified by Polyanskaya I.S., 2005):

- Macroelements (К – 65 000, Ca – 10 000, Mg-9100);
- Millielements (P – 4000, Na – 4300, Al – 460, Fe – 280, Mn – 53, Zn – 47, Sr-47, Rb-39, B – 32);
- Microelements (Ti-13, Cu 6.9, Ni-4.10,Li-2.10, Mo-1.40, Cr-1.20,Ag-1.10, Se-0.50, Ce-0.32, Ga-0.170, Y-0.150, Nd-0.130, Sb-0.110, Sn-0.076, Th-0.071, Zr-0.067, Pt-<0.05, Au-<0.05, Pr-0.04, Sm-0.028
- Nanoelements (Gd-0.18, Ta-<0.01, Tl-<0.01, Eu-0.01, Dy-0.01, Er-0.01, Yb-0.01, Tb-<0.01, Tm-<0.01, Hf-<0.01, Be-0.006, Ho-0.006, In-0.001)

The results indicate that the “Hyposedaf” dry extract has a high biological value, containing substantial amounts of elements crucial for human life, such as potassium, calcium, phosphorus, magnesium, sodium, aluminum, iron, etc. Literature suggests that potassium compounds are vital for muscle contraction and brain function, and their deficiency can lead to fatigue and overall body weakness.

Magnesium is an essential element found in all body tissues, crucial for the normal functioning of cells. It holds particular importance for internal organs, especially the heart and blood vessels.

Calcium compounds present in the analyzed dry extract are key microelements for human life and health. In medical applications, they serve as sedatives, hemostatics, treatments for allergic diseases, and blood pressure regulators. Calcium is key in strengthening the skeletal system, forming tooth enamel, and plays significant roles in the immune system, skin, hair, nail health, and reproductive function. It is also instrumental in enhancing metabolic processes within the body.

The high concentration of sodium compounds in the dry extract, combined with potassium, aids in maintaining acid-base balance, osmotic concentration of blood, activation of numerous enzymes, and contributes to the formation of membrane potential.

Therefore, the “Hyposedaf” dry extract can be regarded as a valuable source of both macro and microelements.

According to normative documents, the content of heavy metals and arsenic in plants and their derived medicinal preparations is in compliance with the standards of XIV State Pharmacopoeia and Sanitary-epidemiological rules and norms. It has been verified that these concentrations do not exceed established limits.

The obtained results are presented in Table 2.

Table 2

Amount of heavy metals and arsenic in dry extract of “Hyposedaf” mkg/g

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

Element	Quantity according to the XIV State Pharmacopoeia	Quantity as requested by the World Health Organization	Determined amount
Lead, Pb	6,0	10,0	0,750
Cadmium, Cd	1,0	0,3	0,012
Mercury, Hg	0,1	-	not identified
Arsenic, As	0,5	1,0	0,10

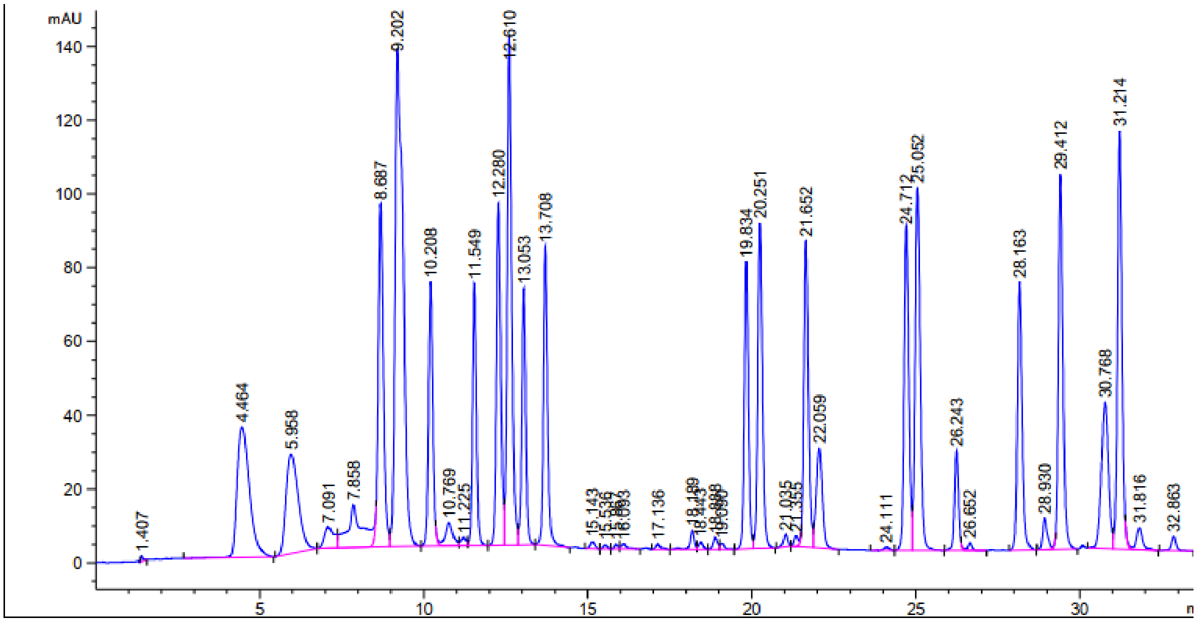
In the subsequent stage of the research, we focused on analyzing the amino acid composition of the "Hyposedaf" dry extract. Initially, the process involved isolating free amino acids. For this, 1 ml of the test sample was combined with an equal volume (1 ml) of 20% trichloroacetic acid. After a duration of 10 minutes, the resultant precipitate was separated through centrifugation at a speed of 8000 revolutions per minute for 15 minutes. Subsequently, 0.1 ml of the clarified liquid was isolated and subjected to lyophilization. The hydrolyzate was then evaporated, and the dry residue was dissolved in a triethylamine-acetonitrile-water mixture (ratio 1:7:1) and dried again. The amino acid phenylthiocarbamyl derivatives (FTC) were synthesized using the phenylisothiocyanate reaction, following the methodology of Steven A. and Koen Daniel. The identification of these amino acid derivatives was carried out via high-performance liquid chromatography (HPLC). Employing an Agilent Technologies 1200 HPLC column detector (75x4.6 mm), we utilized solution A: 0.14M CH3COONa with 0.05% TEA at pH 6.4, and solution B: CH3CN. The flow rate was maintained at 1.2 ml/min, with detection at 269 nm. The gradient percentage per minute was established as follows: 1-6%/0-2.5 min; 6-30%/2.51-40 min; 30-60%/40.1-45 min; 60-60%/45.1-50 min; 60-0%/50.1-55 min [13].

The chromatographic analysis of both the standard amino acid sample of the "Hyposedaf" dry extract and the amino acids present in the extract under study are depicted in Figures 1 and 2.

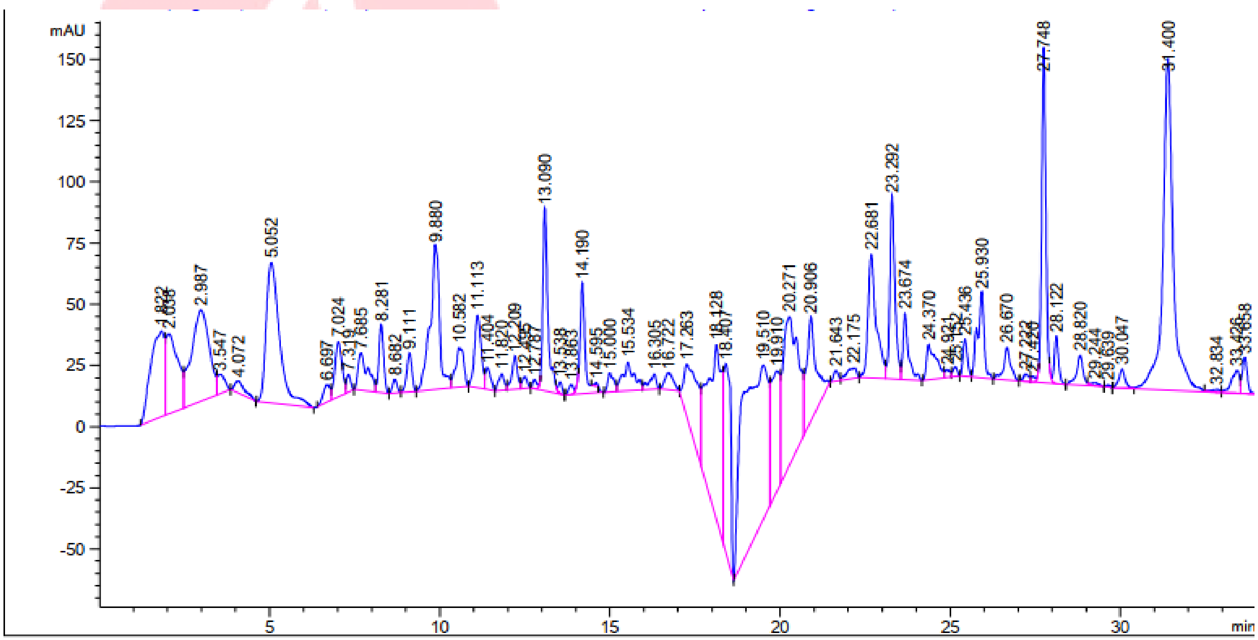
Table 3 presents the quantitative composition and chemical structure classification of the amino acids.

According to the data in Table 3, the "Hyposedaf" dry extract contains 20 amino acids, including 10 essential amino acids, which highlight its substantial quantity and pharmacological significance. The total amino acid content is 29.151 µg/g, of which 15.605 µg/g are essential amino acids, and the remaining 15.605 µg/g are non-essential.

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services



1.Fig. Standard sample chromatogram of a mixture of amino acids



2.Fig. Chromatogram of dry extract of "Hyposedaf"

In the "Hyposedaf" dry extract, histidine stands out as a crucial essential amino acid, present at a concentration of 4.856877 µg/g. It is a key component of hemoglobin — the vital protein in red blood cells responsible for carrying oxygen from the lungs to body cells, and for transporting carbon dioxide in the

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5.705) (2022: 5.705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

opposite direction. A deficiency in histidine can impede the body's ability to produce the necessary quantity of hemoglobin, potentially leading to anemia. Histidine plays an important role in the functioning of the gastrointestinal tract, as well as in liver and kidney operations. Furthermore, the body converts histidine into histamine, a compound integral to the nervous system. Histamine is involved in synthesizing various hormones and is essential for regulating the heart's rhythm [14].

Table 3

Analysis of amino acids of dry extract of "Hyposedaf"

№	Amino acid	Amount of amino acids, mkg/g
Aliphatic amino acids		
Monoaminocarbons		
1.	Alanine	0,077593
2.	Glysine	0,8991
3.	Valin	2,49772
4.	Isoleucine	0,342585
5.	Leucine	1,502734
Oxysaminocarbons		
6.	Serine	0,845982
7.	Threonine	0,310199
Monoaminodicarbons		
8.	Aspartic acid	0,321104
9.	Glutamic acid	0,31053
Monoaminocarbon amides		
10.	Asparagine	1,800129
11.	Glutamine	0,909695
Diaminocarbons		
12.	Lysine	0,194589
13.	Arginine	0,472063
Sulfur preservative		
14.	Cysteine	4,444809
15.	Methionine	2,508621
Aromatic amino acids		
16.	Phenylalanine	0,341222
17.	Tyrosine	3,820187
18.	Tryptophan	0,517421
Heterocyclic amino acids		
19.	Proline	2,178288
20.	Histidine	4,856877
Amount of non-essential amnio acids		13,544

American Journal Of Biomedical Science & Pharmaceutical Innovation
(ISSN – 2771-2753)
VOLUME 04 ISSUE 01 PAGES: 102-112
SJIF IMPACT FACTOR (2021: 5. 705) (2022: 5. 705) (2023: 6.534)
OCLC – 1121105677
Publisher: Oscar Publishing Services

Amount of exchangeable amino acids	15,605
Total amount of amino acids	29,15145

Methionine, at a concentration of 2.508621 μg/g, ranks second in abundance in the "Hyposedaf" dry extract. This essential sulfur-containing amino acid is known for its multiple unique functions [15]. Crucially involved in protein synthesis, methionine's fat-soluble properties also aid in preventing fatty liver disease. Furthermore, it serves as a foundational component for several hormones, including adrenaline, choline, and melatonin. Cysteine, present at 4.444809 μg/g, is another sulfur-bearing amino acid. Classified as semi-essential or conditionally essential, cysteine is unique among the twenty amino acids for containing a thiol group (-HS) capable of undergoing both oxidation and reduction reactions. The oxidation process transforms cysteine into cystine, and it also contributes significantly to metabolic processes and the formation of peptides and proteins [16].

Tyrosine, found at 3.820187 μg/g, plays roles in reducing appetite, decreasing fat accumulation, stimulating melanin production, and enhancing the functionality of the adrenal, thyroid, and pituitary glands [17].

CONCLUSION

In conclusion, the "Hyposedaf" dry extract contains 61 micro and macro elements. This includes 7 essential elements (Co, Cr, Cu, Fe, Mn, Se, Zn) and 4 conditionally necessary ones. Key elements like potassium, calcium, sodium, sulfur, iron, magnesium, and phosphorus are crucial for human health and are prominently featured in the extract. The concentrations of heavy metals and arsenic in the extract were found to be within acceptable limits as defined by the Sanitary-epidemiological rules and norms of State pharmacopoeia of the Republic of Uzbeksitan (Volume 1, Issue 1) and XIV State Pharmacopoeia (RF).

Moreover, the amino acid analysis of the dry extract revealed the presence of 20 amino acids, including 10 essential ones. The total amino acid content is 29.151 μg/g, with essential amino acids accounting for 15.605 μg/g and non-essential ones making up the remaining 15.605 μg/g. These findings underscore the "Hyposedaf" dry extract's richness in amino acids, macro, and microelements, affirming its significant role in the development of other effective pharmaceutical formulations.

REFERENCES

1. 2.3.2.1078-01. Hygienic requirements for the safety and nutritional value of food products. Sanitary and epidemiological rules and standards: [implemented 01.09.2002]. Moscow: Ministry of Health of Russia, 2002, p.266.

2. State Pharmacopoeia of the Republic of Uzbekistan. Tashkent, 2021. Volume 1, Parts 1-2, p.211.
3. GPM 1.2.2.2.0012 Heavy Metals. State Pharmacopoeia of the Russian Federation. XIV edition. Volume II. Moscow, 2018.
4. Davitavyan N.A., Sampiev A.M. Study of the amino acid and mineral composition of the dry extract from field thistle grass // Journal of Health and Education. 2017, vol.19, no.10.
5. Georgievsky V.P., Zinchenko A.A., Kulikov A.Yu., Litvinenko V.I., Kolisnyk A.V., Popova N.V., Bobritskaya L.A. Assessment of immortelle sand flowers for the content of biologically active compounds. Pharmacome // On the standardization of medicinal plant raw materials in the creation of phytopreparations. 2017; 3: pp.34–57.
6. Moricawa T., Ninomiya K., Akaki J., Kakihara N., Kuramoto H., Matsumoto Y., Hayakawa T., Muraoka O., Wang L.-B., Wu L.-J., Nakamura S., Yoshikawa M., Matsuda H. Dipeptidyl peptidase-IV inhibitory activity of dimeric dihydrochalcone glycosides from flowers of Helichrysum arenarium // Journal of Natural Medicines. 2015; 69 (4): 494–506. DOI: 10.1007/s11418-015-0914-8.
7. Skvortsova V.V., Navolokin N.A., Polukonova N.V., Manaenkova E.V., Pankratova L.É., Kurchatova M.A., Masliakova G.N., Durnova N.A. Antituberculous in vitro activity of Helichrysum arenarium extract // Eksp Klin Farmakol. 2015; 78 (2): 30–3.
8. Kariyeva E.S., Gaipova N.N., Nuridullaeva K.N. Study of the amino acid and elemental composition of the complex dry extract "Fitoinflam" // Chemistry of Plant Raw Materials. 2021. No.4. pp.217–223.
9. Kariyeva E.S., Sadikova R.K., Karimov O.U., Maksudova F.X. Study of the amino acid and elemental composition of the dry extract of Samarkand immortelle // Pharmacy. 2022. Vol.71 (8). pp.40–47.
10. State Pharmacopoeia of the Russian Federation. XI edition. 2nd issue. Moscow, 1989.
11. Determination of chemical elements in biological media and preparations by methods of atomic emission spectrometry with inductively coupled plasma and mass spectrometry with inductively coupled plasma: Methodological guidelines. Moscow: Federal Center for State Sanitary and Epidemiological Surveillance of the Ministry of Health of Russia, 2003.
12. Steven A., Cohen Daniel J. Amino acid analysis utilizing phenylisothiocyanate derivatives // Jour. Analytical Biochemistry – 1988. – V.17.-№1.-P.1-16.
13. Histidine: Importance for children and adults.

14. The Role of Methionine.
15. Ismail, N. I. Production of Cysteine: Approaches, Challenges, and Potential Solution [N.I. Ismail, Y. Z. H.-Y. Hashim, P. Jamal] // International Journal of Biotechnology for Wellness Industries. 2014. Vol.3, no.3. pp.95–101.
16. Chief editor Yu.S. Osipov. Tyrosine //[in 35 volumes]. Moscow: Great Russian Encyclopedia, 2004–2017.