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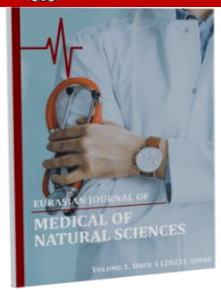

ANALYSIS OF AMINO ACIDS IN THE DRY EXTRACT OF SILYBUM MARIANUM SEEDS

N.A. Akbarov
F.S. Kayumov
G.G. Qayumova
Z.U. Mamatkulov
Tashkent Pharmaceutical Institute, 45 Oybek Street, Tashkent, 100015 (Uzbekistan) e-mail: mzu_77@mail.ru
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ABSTRACT

The study of amino acids in the dry extract of Silybum marianum seeds was carried out. As a result of the conducted research, using high-performance liquid chromatography (HPLC), it was determined that the dry extract contains 20 amino acids, of which 8 are essential amino acids. This composition indicates the high biological value of the extract. The obtained results demonstrate the potential of the dry extract of Silybum marianum seeds for further scientific investigations.

Relevance of the Study

In our Republic, special attention is consistently paid to fully meeting the needs of the population and healthcare institutions for medicines. Our country has a centuries-old rich tradition of treating various diseases with medicinal plants. Based on the invaluable heritage recommended for use in medical practice by our great scholars such as Al-Beruni, Al-Khwarizmi, and Abu Ali ibn Sina, today, the Institute of Bioorganic Chemistry, the Institute of the Chemistry of Plant Substances of the Academy of Sciences of Uzbekistan, the Tashkent Pharmaceutical Institute, as well as several other higher educational institutions, have developed scientifically grounded and clinically proven medicinal plant preparations that have found their place in modern medical practice. At present, various biologically active substances, mineral compounds, proteins, and amino acids are widely used for the normal functioning of the human body. Amino acids are particularly noteworthy for their broad therapeutic effects and their ability to enhance the absorption of other substances in the body, which highlights their vital role in human life. Just as proteins and amino acids form the basis of living nature, they also constitute the foundation of our dry extract with choleretic properties [1, 2, 3].

Research Objective: To analyze the amino acids of the dry extract of *Silybum marianum* seeds using modern methods.

Materials and Methods. For the analysis of amino acids, an Agilent Technologies 1200 chromatograph equipped with a 75 × 4.6 mm Discovery HS C18 column was used [5, 6]. For mobile phase “A”: 0.14 M CN_3COONa + 0.05% TEA (triethylamine), pH 6.4. For mobile phase “B”: acetonitrile for HPLC was applied.

Chromatography conditions: flow rate – 1.2 ml/min; column temperature – 25 °C; wavelength – 269 nm; injection volume – 5 µl. The results are presented in

Table 1.

Mobile phase gradients:

Time, min	Mobile phase B, %	Mobile phase A, %
0-2.5 **min**	1-6%	99-94%
2.51-40 **min**	6-30%	2
40,1-45 **min**	30-60%	2
45,1-50 **min**	60%	30
50,1-55 **min**	60-0%	30

The test samples and standard substances were dissolved in ethanol and filtered through a Millipore filter with 0.2 µm pores.

Preparation of working standard solution (WSS): The following amino acid standards were used: aspartic acid (Asp), asparagine (Asn), glutamic acid (Glu), glutamine (Gln), seroproline (Gl.Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), methionine (Met), cystine (Cys_2) and cysteine (Cys). In addition, purified water, high-purity acetonitrile, high-grade isopropyl alcohol, sodium acetate, phenyl isothiocyanate, hydrochloric acid, and sodium hydroxide were applied. The amino acids were dissolved in a concentrated solution of 1 mol/L hydrochloric acid and dried at 65 °C in an air stream passing through a capillary in the pump. To the dried residue, 0.10 ml of 0.15 mol/L sodium hydroxide solution was added and thoroughly mixed. Subsequently, 0.35 ml of phenyl isothiocyanate solution in isopropyl alcohol was introduced, mixed, and followed by the addition of 0.05 ml of purified water. When turbidity appeared, the test tube was heated in a water bath at 60 °C for 10–15 seconds until the solution became clear. The mixture was then kept at room temperature for 20 minutes and immediately dried again in a water bath at 60 °C for 10–15 minutes. The resulting dry residue was dissolved in 1 ml of purified water and filtered through a membrane filter with a pore size of 0.45 µm. The prepared solutions were consecutively injected into the chromatographic column (Figures 1–2).

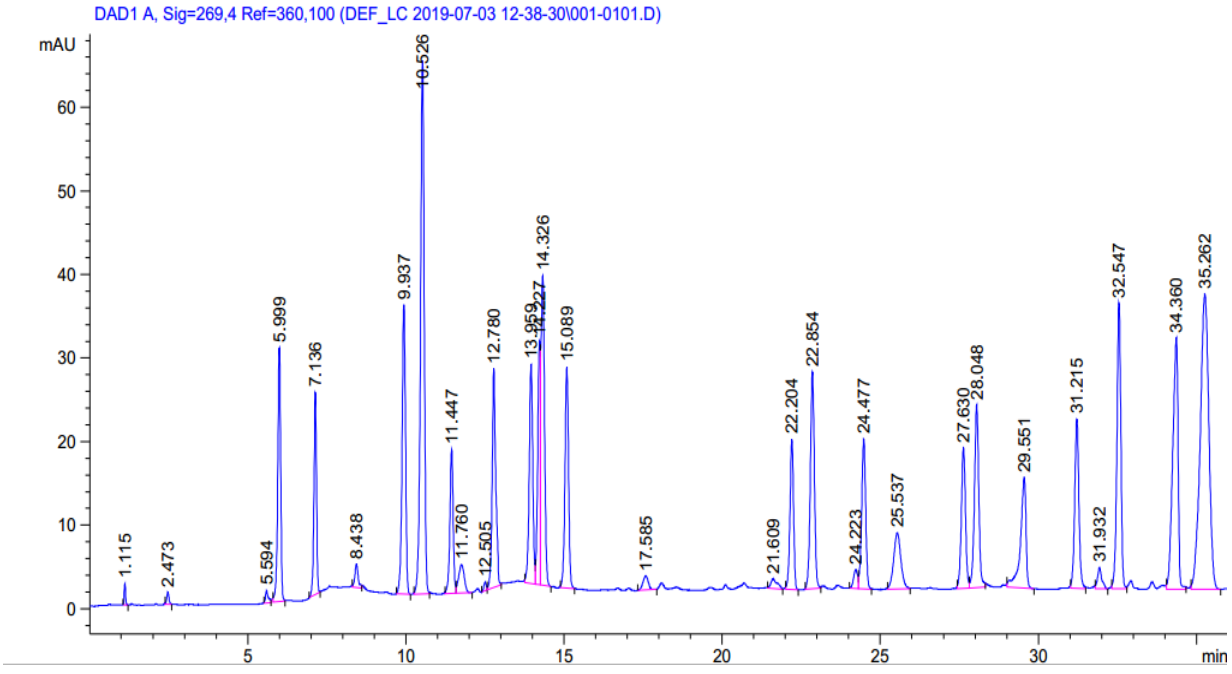

Figure 1. Amino acid peaks formed in the chromatogram

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Preparation of the test sample. The protein and peptide aqueous extract was precipitated in a centrifuge tube. For this, 1 ml (accurate volume) of 20% trichloroacetic acid was added to 1 ml of the test sample. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. 0.1 ml of the precipitate was separated, freeze-dried and filtered. The resulting solutions were filtered through a 0.2 μm pore size Millipore filter and chromatographed under the above conditions.

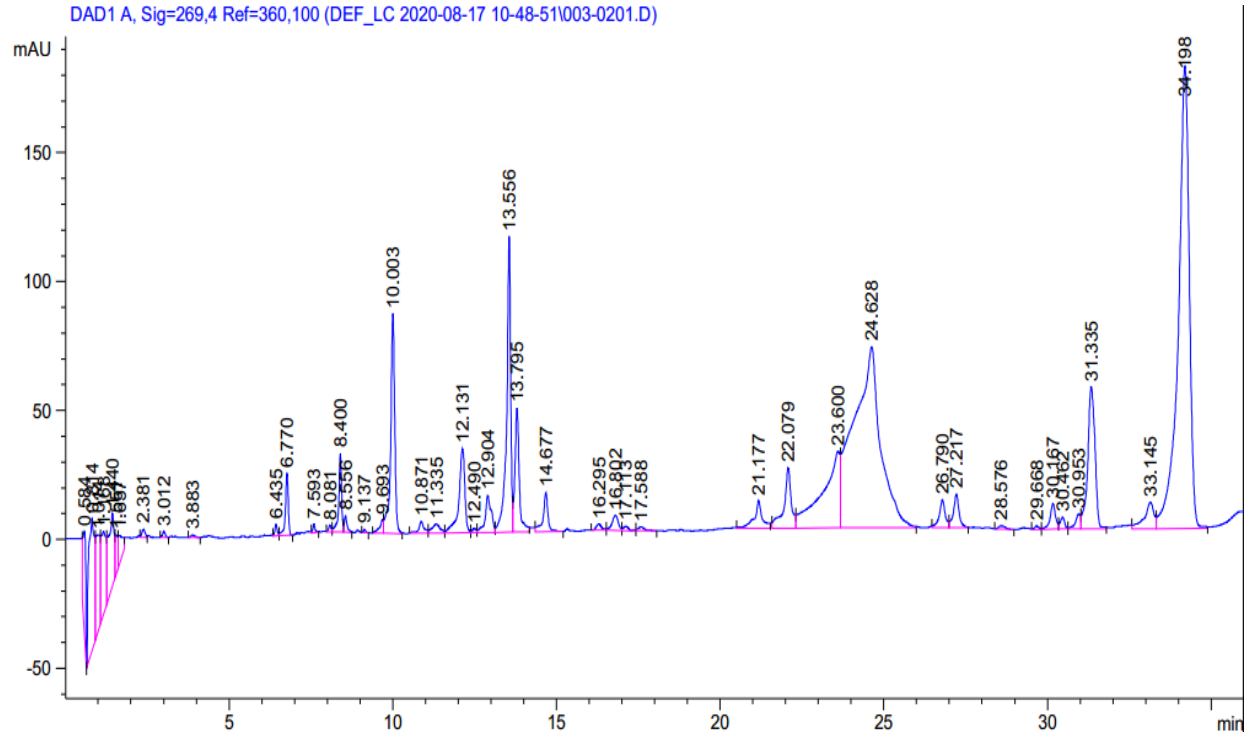

Figure 2. Amino acid chromatography of dry extract silybum marianum

Results and Discussion

As a result of the analysis, it was determined that the dry extract of Silybum marianum contains 20 amino acids identified by High-Performance Liquid Chromatography (HPLC), of which 8 are essential amino acids: tryptophan, phenylalanine, methionine, lysine, valine, threonine, isoleucine, and leucine. The amino acids present in the dry extract of of Silybum marianum were classified into four groups according to their structural characteristics (Table 2).

Table 2

Analysis of dry extract of milk thistle seeds amino acids

amino acid name	Milk thistle mg/ g	amino acid name	Milk thistle mg/ g
Aspartic k-ta	0.034	Glutamine	0,19
Glutamic k-ta	0.314	Cysteine	0.35
Serene	0.052	Threonine	0.47
Glycine	0.56	Argenine	0.35
Asparagine	0.48	Alanine	0.39
Proline	0.31	Leucine	0.29

Tyrosine	0.43	Histidine	0,07
Valin	0.76	Tryptophan	0.18
Methionine	0.39	Phenylalanine	0.12
Isoleucine	0.29	Lysine HCl	0,18

In Table 2, along with the retention times of the amino acid samples, the peak areas of both the standard and test samples are presented, and their quantities were calculated using the corresponding formula.

A method for the qualitative and quantitative determination of amino acids in the dry extract of Silybum marianum using HPLC was developed. The analysis was performed in five replicates, and the method was subjected to metrological evaluation with respect to the total amino acid content. The results of the metrological analysis are presented in Table 3.

Table 3.

Metrological Analysis of Amino Acid Quantification by HPLC

№	Total Amino Acid Content, mg/g	Results of Metrological Analysis
1.	72,50266	X_{av}=72,50255 S^2=0,00122 S=0,02937 Δx=0,03733 ye=0,10950
2.	72,50245	
3.	72,50235	
4.	72,50265	
5.	72,50295	

The analysis showed that the amino acids in the Silybum marianum dry extract are present at a concentration of **72.50225 ± 0.2 mg/g**.

Conclusion: For the first time, using High-Performance Liquid Chromatography (HPLC), **20 amino acids** were identified in the Silybum marianum dry extract, of which **8 are essential amino acids** (tryptophan, phenylalanine, methionine, lysine, valine, threonine, isoleucine, and leucine).

Moreover, certain amino acids were found in particularly high amounts in the Silybum marianum dry extract: **Glutamic acid** (2-aminopentanedioic acid) is a non-essential aliphatic dicarboxylic amino acid. It is an integral part of proteins in all living organisms, participates in the metabolism of nitrogen-containing biochemical compounds, in protein-carbohydrate metabolism, and in the synthesis of other amino acids. **Asparagine** (aminoacyl acid, $HO_2CCH(NH_2)CH_2CO_2H$) is abundant in animal and plant proteins and plays an important role in the metabolism of nitrogenous compounds. It also participates in the formation of pyrimidine bases and in urea synthesis. Since it exists both freely and as part of proteins in all organisms, it can be synthesized artificially. Proline (α-pyrrolidinecarboxylic acid, $C_5H_9O_2N$) is found in all proteins. It is abundant in prolamins (from cereal grains), collagen, κ-casein, and elastin. Proline is also present in biologically significant peptides such as insulin, adrenocorticotropic hormone, gramicidin S, and others. Histidine (α-amino-β-5-imidazolylpropionic acid, $C_6H_9O_2N_3$) is a crystalline substance. When metabolized in the body, it can produce histamine (upon

decarboxylation), ammonia, glutamic acid, and formic acid (upon deamination). Medically, histidine is used in the treatment of stomach and duodenal ulcers. The diverse and sufficiently high content of physiologically active amino acids indicates that they participate in the complex systemic effects of raw materials on the whole organism, helping to normalize the functions of multiple systems.

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