

AMINO ACID ANALYSIS OF DRY EXTRACT OF MILK THISTLE FRUIT SILYBUM MARIANUM GAERTN L. GROWING IN UZBEKISTAN

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ABSTRACT:

Information about amino acids synthesized by plants, their functions in amino acid metabolism, the pharmacological activity of some of them, especially in the formation of hepatoprotective factors of the body's defense in liver diseases, is generalized. This article presents the amino acid composition of dry extracts of plant seeds grown in the environment of Uzbekistan, describes the analytical results obtained using the HPLC (High Performance Liquid Chromatography) method, and their conclusions. The object of the study was the fruits of the milk thistle [*Silybum marianum* (L.) Gaertn.]

KEYWORDS: Amino acid, analysis, HPLC method, milk thistle, dry extract, vitamins, physiology, and medicinal plant.

INTRODUCTION:

Recently, there has been an active search for drugs that increase the liver's resistance to the pathological effects of xenobiotic. Various pharmacological agents that normalize metabolic processes in the body, as well as compounds with antioxidant activity

and inhibiting lipid peroxidation, can exhibit a hepatoprotective effect to one degree or another. Preparations based on flavolignans from milk thistle are widely used, which improve the detoxifying function of the liver by stabilizing hepatocyte membranes, increasing the activity of antioxidant enzymes, as well as enzymes of the first and second phases of detoxification [1]. A number of such properties, such as their effect on human physiology and lifestyle, are directly related to amino acids, which make up the basic structural unit of the protein of this plant. Amino acid analysis was carried out to study the order and degree of their effect on the human body.

The aim of our research was to determine the amino acid composition of the dry extract of the fruit of the milk thistle *Silybum marianum* Gaertn L.

MATERIALS AND METHODS:

The precipitation of proteins and peptides of the aqueous extract was carried out in centrifuge beakers. For this, 1 ml (exact volume) of 20% trichloroacetic acid (TCAA) was added to 1 ml of the test sample. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Separating 0.1 ml of the sedimentary liquid,

freeze-dried. HPLC analysis of phenylthiocarbamyl (FTC) -derivatives of free amino acids. The synthesis of FTC (phenylthiocarbomail) derivatives of free amino acids was carried out according to the method of Steven A., Cohen Daviel. The identification of FTC amino acids is carried out on an Agilent Technologies 1200 chromatograph on a 75 x 4.6 mm Discovery HS C 18 column. Solution A: 0.14 M CH₃COONa + 0.05% TEA pH = 6.4 B: CH₃CN: CH₃OH (2: 3) ... Flow rate 1.2 ml / min, absorption 269 nm. [12]

EXPERIMENTAL PART:

Isolation of free amino acids. Precipitation of proteins and peptides of the aqueous extract in centrifuge beakers. For this, 1 ml (exact volume) of 20% TCA was added to 1 ml of the test sample. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Separating 0.1 ml of the sedimentary liquid, freeze-dried.

HPLC analysis of PTK derivatives of amino acids. The synthesis of FTC

(phenylthiocarbomail) derivatives of free amino acids was carried out according to the method of Steven A., Cohen Daviel. The identification of FTC amino acids is carried out on an Agilent Technologies 1200 chromatograph on a 75x4.6 mm Discovery HS C18 column. Solution A: 0.14M CH₃COONa + 0.05% TEA pH 6.4, B: CH₃CN. Flow rate 1.2 ml / min, absorption 269nm. Gradient% B / min: 1-6% / 0-2.5min; 6-30% / 2.51-40min; 30-60% / 40.1-45min; 60-60% / 45.1-50min; 60-0% / 50.1-55min.

RESULTS AND DISCUSSION:

The research objects were dry extract of milk thistle fruit. The study of the amino acid composition was carried out according to the above method. The results of studies of the amino acid composition of dry extract, obtained on the basis of dry extract of milk thistle fruit, are shown in table1.

Table1 Analysis of dry extract of milk thistle amino acid seeds

Amino acid name	Silybum marianum mg/gr	Amino acid name	Silybum marianum mg/gr
Aspartic acid	0,044849	Glutamine	0,192853
Glutamic acid	0,414607	Cysteine	0,372845
Serine	0,062133	Threonine	0,590935
Glycine	0,568913	Argenin	0,40293
Asparagine	0,58066	Alanin	0,64546
Proline	0,309895	Leucine	0,339664
Tyrosine	0,519465	Histidine	0,086226
Valine	0,680075	Tryptophan	0,265141
Methionine	0,44509	Phenylalanine	0,565292
Isoleucine	0,329058	Lysine HCl	0,204705

1-graphs Analysis for vitamins

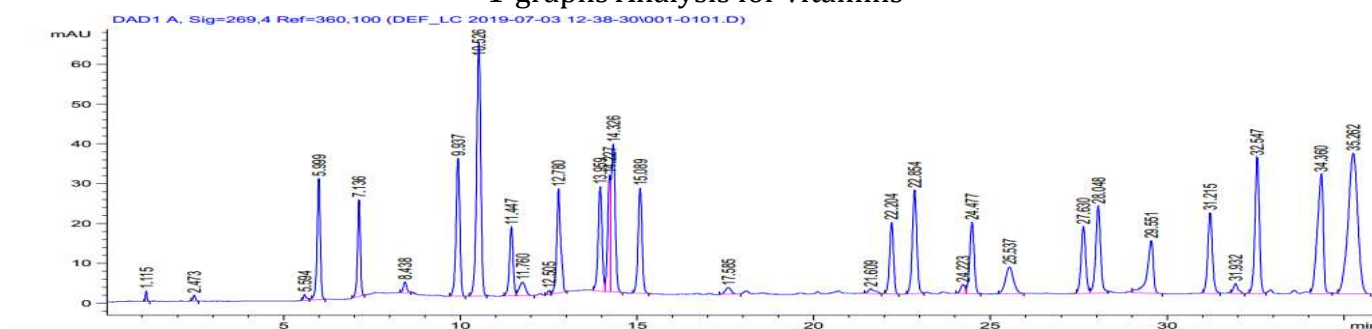


Fig-1. Chromatogram of the vitamin composition of dry extract of milk thistle fruitAcq.

Operator: Seq. Line: 1; Acq. Instrument: Instrument 1 Location: Vial 1; Injection Date: 7/3/2019 12:38:49 PM Inj : 1; Inj Volume : 5.0 µl; Acq. Method: C:\CHEM32\1\DATA\DEF_LC 2019-07-03 12-38-30\AAA_LC.M; Last changed: 7/3/2019 11:58:45 AM; Analysis Method: C:\CHEM32\1\METHODS\VITAMINS_LC.M; Last changed: 6/21/2019 10:13:06 AM; Method Info: Vit. Bufer. 0.5% Uksus.k-ta: MeC

The results of studies of the vitamin composition of dry extract obtained on the basis of dry extract of milk thistle fruit are shown in table 2

Table 2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.115	BB	0.0488	7.55833	2.53101	0.1466
2	2.473	BB	0.0759	7.43866	1.49873	0.1443
3	5.594	BB	0.0876	9.29058	1.51337	0.1802
4	5.999	BB	0.0987	194.95033	30.45720	3.7814
5	7.136	BB	0.0859	137.04053	24.24963	2.6582
6	8.438	BB	0.1095	20.91950	2.85512	0.4058
7	9.937	BB	0.1158	261.04593	34.69249	5.0635
8	10.526	BB	0.1139	525.93903	63.82017	0.2016
9	11.447	BV	0.1183	125.54469	17.26083	2.4352
10	11.760	VB	0.0868	42.21429	3.39016	0.8188
11	12.505	BV	0.1140	5.63540	1.03821	0.1093
12	12.780	VB	0.1193	206.75104	26.24187	4.0103
13	13.959	BV	0.1818	203.14856	26.23913	3.9404
14	14.227	BB	0.1935	167.26015	29.21017	3.2443
15	14.326	BB	0.1189	286.22836	37.09195	5.5519
16	15.089	BB	0.1375	207.28357	26.48636	4.0207
17	17.585	BB	0.1323	19.69217	1.69160	0.3820
18	21.609	BB	0.1354	16.20948	1.15646	0.3144
19	22.204	BB	0.2393	140.19983	18.00401	2.7194
20	22.854	BB	0.1423	230.51587	25.99416	4.4713
21	24.223	BV	0.1323	19.64561	2.24093	0.3811
22	24.477	VB	0.1354	159.16437	17.96035	3.0873
23	25.537	BB	0.2393	105.40646	6.71050	2.0446
24	27.630	BV	0.1423	153.12567	16.80270	2.9702
25	28.048	VB	0.1456	209.03886	21.86107	4.0547
26	29.551	BB	0.1794	163.08650	13.26938	3.1634
27	31.215	BB	0.1414	186.70912	20.27953	3.6216
28	31.932	BB	0.1360	23.79462	2.57046	0.4615
29	32.547	BB	0.1353	297.29230	34.23524	5.7665
30	34.360	BB	0.1791	353.71921	30.10885	6.8611
31	35.262	BB	0.2912	669.61804	35.32468	12.9885

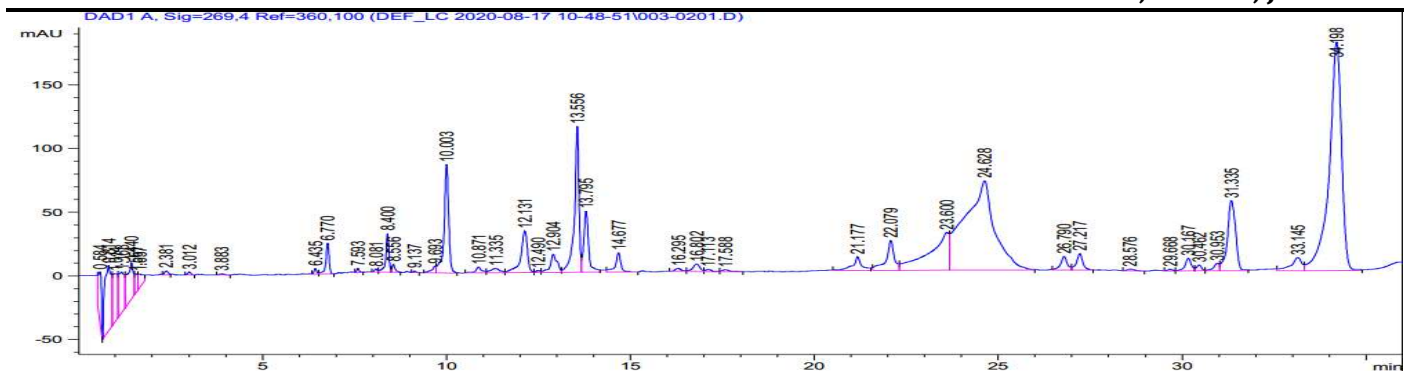


Fig-2. Chromatogram of the amino acid composition of dry extract of milk thistle fruit

Acq. Operator: Ishimov U.J. Seq. Line: 2; Acq. Instrument: Instrument 1 Location: Vial 3; Injection Date: 8/17/2020 11:28:48 AM In: 1; Inj Volume: 30.0 µl; Acq. Method: C:\CHEM32\1\DATA\DEF_LC 2020-08-17 10-48-51\AAA_LC.M; Last changed: 8/17/2020 10:48:50 AM by Ishimov U.J.; Analysis Method: C:\CHEM32\1\METHODS\STEROIDE.M; Last changed: 8/7/2020 2:33:48 PM by Ishimov U.J.; Method Info: Steroide B: 83%H₂O,

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	0.584	BV	0.0915	246.82047	40.25559	1.4257
2	0.814	VV	0.1595	651.68237	51.53517	3.7644
3	1.014	VV	0.1284	356.13705	36.97183	2.0572
4	1.168	VV	0.1368	338.28027	32.13482	1.9540
5	1.440	VV	0.1695	372.72302	27.95851	2.1530
6	1.551	VV	0.0692	74.54256	15.21388	0.4306
7	1.657	VB	0.1195	95.20549	10.73010	0.5499
8	2.381	VV	0.1165	22.64489	3.05466	0.1308
9	3.012	BV	0.0888	13.73097	2.32706	0.0793
10	3.883	BB	0.1222	8.68444	1.03208	0.0502
11	6.435	BV	0.0749	22.90206	4.53808	0.1323
12	6.770	VB	0.0913	147.56325	24.15274	0.8524
13	7.593	BB	0.0708	16.25276	3.46315	0.0939
14	8.081	BV	0.0828	14.37061	2.58893	0.0830
15	8.400	VV	0.0883	184.12680	30.55730	1.0636
16	8.556	VB	0.0894	38.12611	6.23227	0.2202
17	9.137	VB	0.0777	6.73846	1.31605	0.0389
18	9.693	BV	0.1109	42.33507	5.20625	0.2445
19	10.003	VB	0.1296	742.85376	85.26078	4.2910
20	10.871	BV	0.1580	51.90902	4.58675	0.2998
21	11.335	VV	0.2548	59.89264	3.44635	0.3460
22	12.131	VV	0.1906	414.57001	32.55648	2.3947
23	12.490	VV	0.1157	12.94187	1.57721	0.0748
24	12.904	VV	0.1731	184.34564	14.42016	1.0648
25	13.556	VV	0.1234	978.29205	114.77154	5.6510
26	13.795	VB	0.1345	439.30850	48.13670	2.5376
27	14.677	BB	0.1497	159.07253	15.24454	0.9189
28	16.295	BV	0.1793	28.28651	2.43926	0.1634
29	16.802	VV	0.2030	78.09586	5.88004	0.4511
30	17.113	VB	0.1778	17.37266	1.53722	0.1004

31	17.588	BB	0.2073	20.07792	1.43461	0.1160
32	21.177	BB	0.2260	178.81953	10.64604	1.0329
33	22.079	BV	0.2141	361.77979	23.44134	2.0898
34	23.600	VV	0.4742	1120.34412	29.69073	6.4715
35	24.628	VB	0.7216	3996.23096	70.25337	23.0837
36	26.790	BV	0.1819	133.42303	10.66785	0.7707
37	27.217	VB	0.1759	153.84099	12.83259	0.8886
38	28.576	BB	0.2137	15.94830	1.19481	0.0921
39	29.668	BV	0.1336	10.90729	1.25297	0.0630
40	30.167	VV	0.1715	114.66431	9.86980	0.6623
41	30.462	VV	0.1445	44.45270	4.60798	0.2568
42	30.953	VV	0.1542	62.32272	5.85457	0.3600
43	31.335	VB	0.2455	857.95660	55.14406	4.9559
44	33.145	BV	0.2886	209.73444	10.25330	1.2115
45	34.198	VB	0.3440	4211.61865	179.35448	24.3278

Totals: 1.73119e4 1055.62401

Sorted By: Signal; Multiplier: 1.0000; Dilution: 1.0000; Use Multiplier & Dilution Factor with ISTD;
Signal 1: DAD1 A, Sig=269, 4 Ref=360,100

CONCLUSIONS:

At the end of the study, the required results were obtained, according to which the chemical composition of the dry seed extract was different. The studied milk thistle fruit dry extract is represented by 7 essential and 13 nonessential amino acids. In particular, glycine, asparagine, valine, threonine, alanine, phenylalanine are more abundant than other amino acids. The amount of Asparaginov K-ta and Histidine was much lower, which corresponds to a hundred measurements. All information contained in this article is the result of research carried out at the Research Institute of Bioorganic Chemistry by the laboratory of proteins and peptides in the name of Sadikov.

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