Quantity Of Silybum Growing In Uzbekistan Methods Of Silimarin In The Plant And Methods Of Analysis Of Mass-Spectrometry Of Flavolignanes In Extract

Kayumov Feruz Sobir ugli[1] , Tukhtaev Hakim Rakhmonovich[2],Tukhtaev Farkhad Hakimovich[3] , Ulugmurodov Nor Hudoykulovich[4] ,Foziljonov Shukrullo[5]

[1]Assistant of the department of biotechnology, Tashkent pharmaceutical institute,
[2]Doctor of Pharmaceutical Sciences, Professor, Department of Inorganic, Physical and Colloid Chemistry, Tashkent Pharmaceutical Institute,
[3]Candidate of Pharmaceutical Sciences, Associate Professor. Department of Biotechnology, Tashkent Pharmaceutical Institute,
[4]Candidate of Technical Sciences, Associate Professor, Department of Physics, Mathematics and Information Technology Tashkent Pharmaceutical Institute,
[5] Student of Andijan State University. Uzbekistan.

Emails : [1]kildonferuz@mail.ru, [2]hakimrahmonovich@gmail.com, [3]pharmi@yandex.ru, [4]norhudoykulov@mail.ru, [5]shukrullofoziljonov@gmail.com

Abstract : The chemical composition of growing Silybum marianum in the south-eastern part of Uzbekistan: the amount of silymarin and flavonolignans was studied. Silybum marianum has almost no contraindications and is rich in chemical composition, which positively affects the body and human health. It can normalize the work of a number of body systems in a short time and strengthen the immune systematic, and this directly depends on the amount and concentration of biochemical compounds contained in its chemical composition. We analyzed the dry extract of the seed in HPLC and paid special attention to the number of Flavolignans contained in it. It has been studied that the full effect of flavonolignans contained in Silybum depends on its chemical concentration in terms of importance and stable antioxidant performance. This article describes the results obtained when the taxifolin, Silibin, Silidianin and Silikristin flavonolignans after the results in HPLC were continued in mass spectrometry.

Keywords. chromatographic, dry extract, flavolignan, HPLC-MS, Silybum marianum, silymarin.

1. INTRODUCTION.

Milk thistle (*Silybum marianum*) is a plant originating from the northern parts [P., Bombardier, 1995 E. Marazzani.The Shape of the Silybum in Africa and the Mediterranean] reaches one (in cultured forms) or two-year-old barbed plant bo internet 1-1,5 meters of vital marianum. In Uzbekistan, the growing STEM is simply branched, or without thorns. The leaves are eared consecutively on the stem, ellipsoid, chewed eared, large (go up to 80 cm in length). Along the Leaf and its veins on the surface of the back, yellow Thorns were eying. Have white dogs.

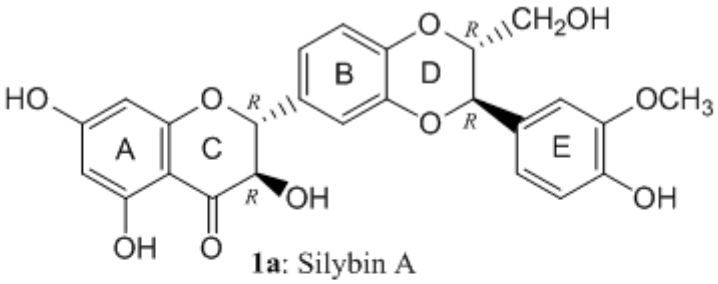
On the surface of the leaf plate green shiny flowers for red, pink or white, which are collected in a large spherical single basket. Around her flower eat thorny leaves. to make the Rose of the rose thick, covered with feathers. All blooms are same-sex, two in a tube. It blooms in July-August.

Part of the main cultivation envisaged for O Uzbekistan is V seed and Ola is O (*Silybum marianum L.*); Seed; typical bo own soils, Plains correspond to land areas, conditionally insured (mesophyte) plant; Book of Kashkadarya region, it is reported that Yakkabag is cultivated [PD-4670 10.04.2020] grown in the regions of O, Boysun, Sariosiya, Uzun districts of Surkhandarya region, Jizzakh, all districts of Tashkent regions.

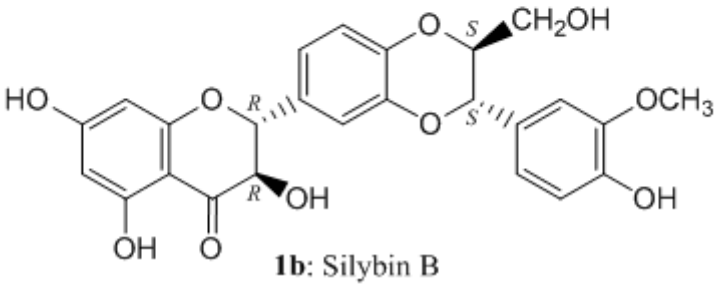
From this euthanasia, the seed is widely used as a hepatoproteinektiv agent in the form of a standardized extract. The activity of Silymarin hepatoprotektiv is anti-inflammatory: it is an antioxidant and can be seen in the actions of the liver and immunities cells [Polyak, S.C., Morishima, M.C., Dev, C.C., Liu, Y., 2007].

In addition, it was found that the active biochemical composition (silibinin in the example during the first World War) does not eat negative effects on the reproduction property of cancer cells [Tyagi, A. K., Phetia, N., Condon, M. S., Basland, M. C., Agerwel, C, Agerwel, R. 2002-the year.]. In the composition of a mixture of eyewitnesses, Flavolignan contains silibinin, isosilbine, and silichristin, which are in the same composition.

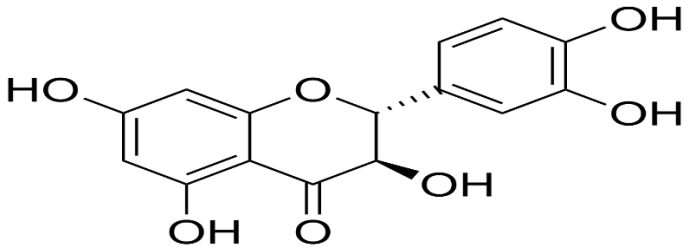
Initially, compounds were absolute and later silibinin isomerizations were determined and a, B were determined in the structure of the configuration spectroscopy [Kim, N., Graf, T. N.Y., Sparacino, C. M., Wani, M. C., Wall, M. E., 2003.] (A; B) currently, during the first World War, Isocylibine Ham has such an isomerization [Fig.1.1]. [Scott J. Brantley, Nicholas X. Oberlies, David J. Kroll and Mary F. Paine.].



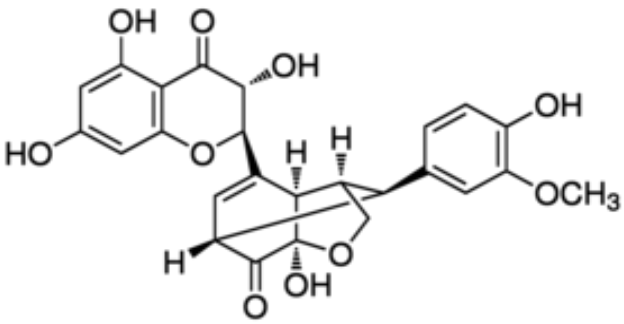
1a: Silybin A



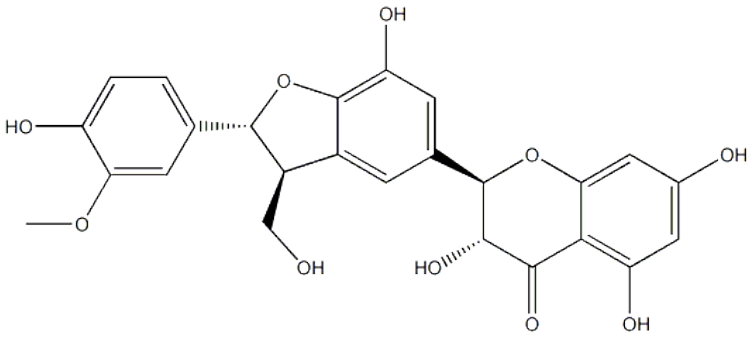
1b: Silybin B



1c: Taxifolin



1d: Silidianin



1e: Silikristin

Fig.1.1 The chemical structure of some flavonolignans of Silymarin

This study was conducted on the Slybum plant growing in Uzbekistan (especially in the Tashkent region) and on its chemical composition. It is known that the chemical composition of plants varies depending on the growing environment. The bio preparations used in the study and their concentrations are also expected to have a direct effect on the results.

2. MATERIALS AND METHODS

2.1. Concentration of instruments and solutions used. Reverse phase nano-LC-MS / MS was performed using an Agilent 1200 nano-flow LC system coupled to an Agilent Technologies 6520B series CHIP-Q-TOF mass spectrometer.

The sample was fractionated using an Agilent Technologies 1200 series chromatograph through a Zorbax SB C18 chip, 5 µm, 75 µm x 43 mm. Mobile phase: A - 0.1% formic acid solution + 5% acetonitrile, B - acetonitrile + 0.1% formic acid + 10% deionized water.

The application was performed on an Agilent Technologies 1260 Cap Pump series instrument at a flow rate of 4 µL / min. Elution was performed on an Agilent Technologies 1260 Nano Pump series instrument at a flow rate of 0.6 µL / min. The concentration gradient of solution B - in minutes: 0% - 3 minutes, 60% - 15-20 minutes, 0% - 24 minutes. The solutions were degassed on an Agilent Technologies 1260 µ-degasser. Samples were loaded onto the column using an Agilent Technologies Micro WPS instrument, 2 µL each.

The eluted fractions were analyzed by mass spectrometry under the following conditions: Ionization source: ESI +, drying gas flow: 4 L / min, drying gas temperature: 350 ° C, the voltage at the skimmer cone: 65V, on a fragmentor 175V, mass range: in MS 300 mode - 3000 m / z, in MS / MS 50 mode - 2500 m / z, with the voltage at CAP in the range 1800-2500V. Support ionization: positive. The units we use in the laboratory are based on the work of Kuki, O., Nagy, L., Deák, G., Nagy, M., Zsuga, M., & Kéki, S. (2011).

2.2. Used plant product and extract

Fig.2.1 Plant seed grown in the study area (**41°18′0″N 69°16′0″E**) and in the study area

№	Amount of defatted seeds, g	Extravagant type	Extravagant mass, ml	Ultrasonic extraction time, min	Amount of silymarin, in%
1	2	purified water	10	15	1,21
2	2	purified water	10	30	1,32
3	2	purified water	10	60	1,58
4	2	70% ethanol	10	15	1,54
5	2	70% ethanol	10	30	1,72
6	2	70% ethanol	10	60	2,95
7	2	96% ethanol	10	15	1,36
8	2	96% ethanol	10	30	2,60
9	2	96% ethanol	10	60	3,32

2.2 **. Table.** Appropriate quantities of products used in the extraction

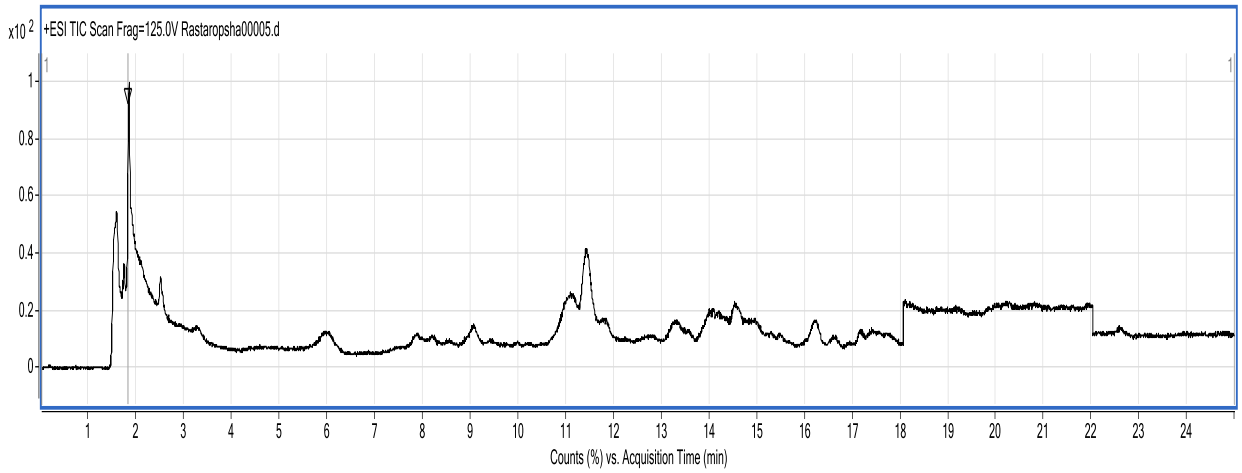
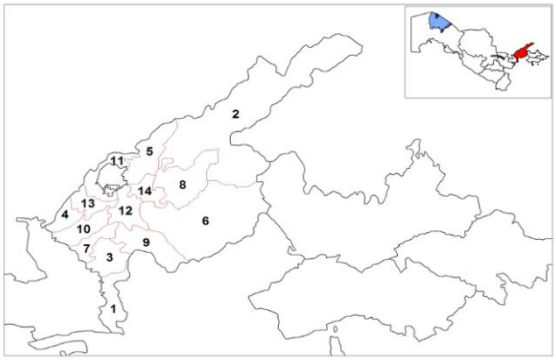
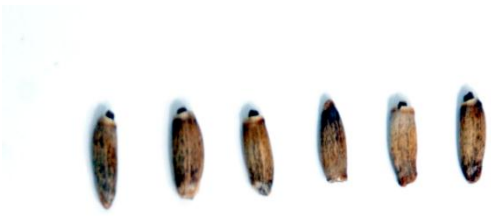


Fig. 2.3. Continuity and status of seed extract chromatography

3. RESULTS AND DISCUSSION





3.1. Sorting seeds for extract

Seeds were collected from 14 regions to obtain *Silybum* seed extract [fig.2.1]. Seeds collected from all over the region were selected by balancing their size and mass to minimize disparities between them.

3.2. Extraction process and indicators

The following are the main technological steps in obtaining a dry extract from the plant *Silybum marianum*:

1. Preparation of raw materials. Plant seeds are inspected, their appearance, storage, size, moisture content are determined, ground in a mill and weighed.

2. Extraction process. It is degreased using extraction gasoline.

3. Separation process. The resulting liquid separation was stopped and the oily part containing the extraction gasoline was separated. The resulting short, ie degreased seeds, were transferred to the next extraction process.

4. Driving process. Extracted gasoline stored in the oily part was separated from the liquid system by driving.

5. Extraction process. Degreased seeds were extracted with 96% ethanol. Hydromodule 1: 5, temperature 400S, extraction time 60 minutes.

6. Separation process. The resulting extract was precipitated, centrifuged to remove ballast, and filtered to remove slag.

7. Evaporation process. The resulting liquid extract was evaporated and the excess liquid was evaporated.

8. Drying process. The dry extract was obtained by drying the evaporated burnt extract in a dryer at a temperature not exceeding 600C and humidity of 5-6% [Table 2.2].

The yield of dry extract from the plant *Silybum marianum* was 15-17%. The resulting dry extract was evaluated for organoleptic and physical appearance. The colour of the extract is a light brown, hygroscopic powder with a characteristic odour.

3.3. The results of the analysis of the standard extract

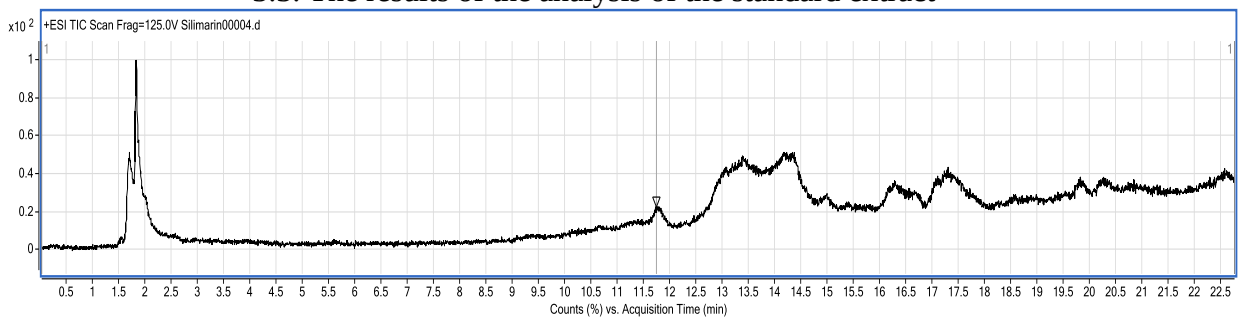
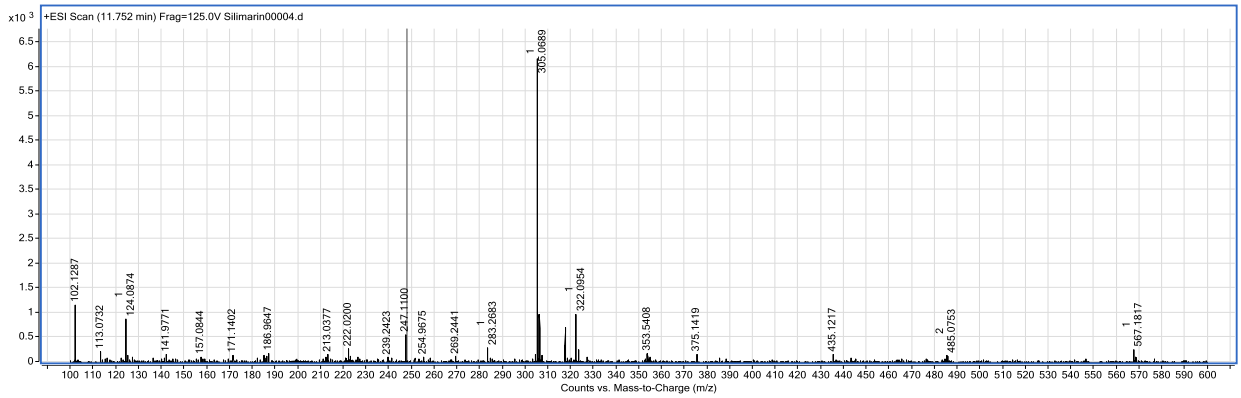


Fig. 3.1. The state of the standard solution over time



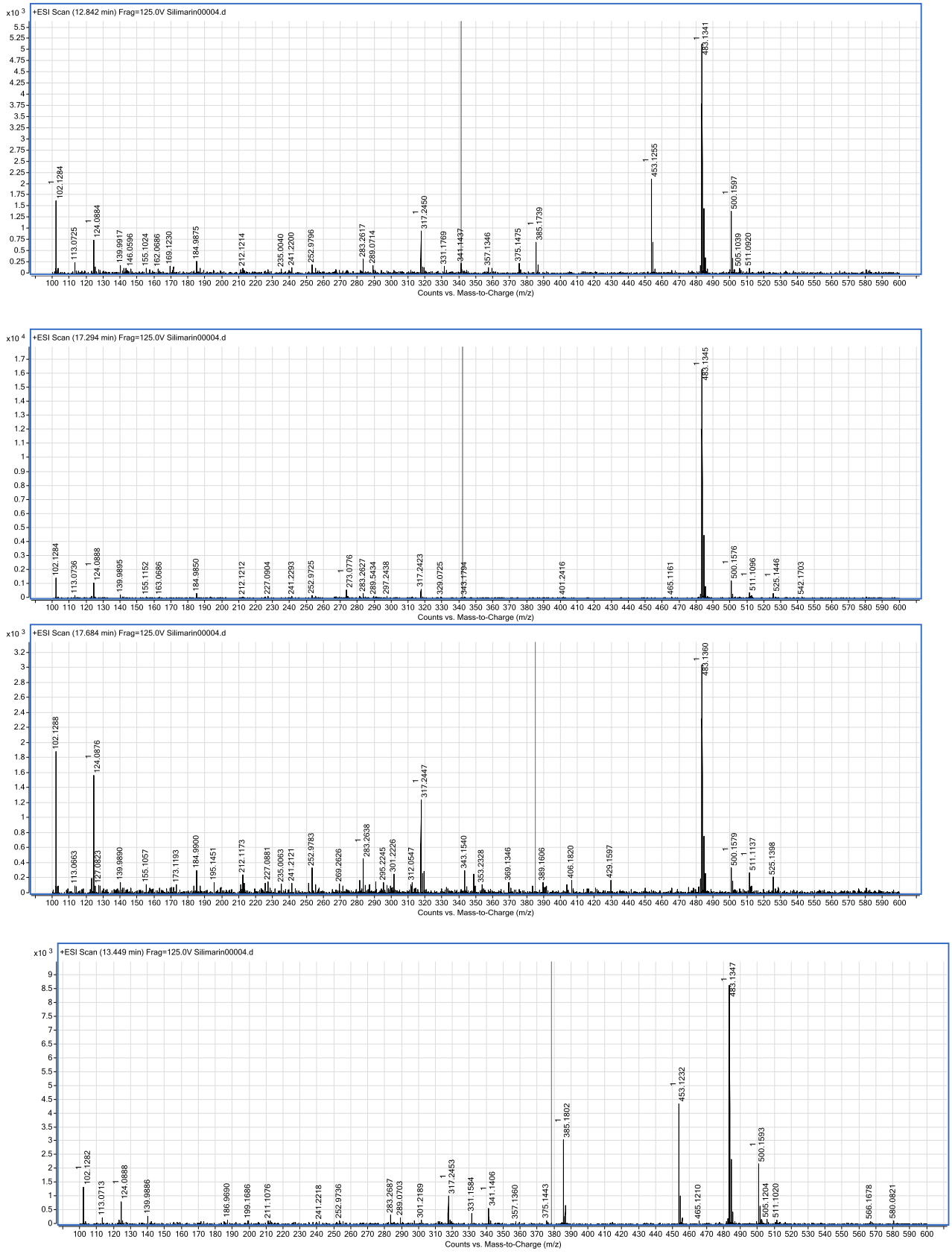
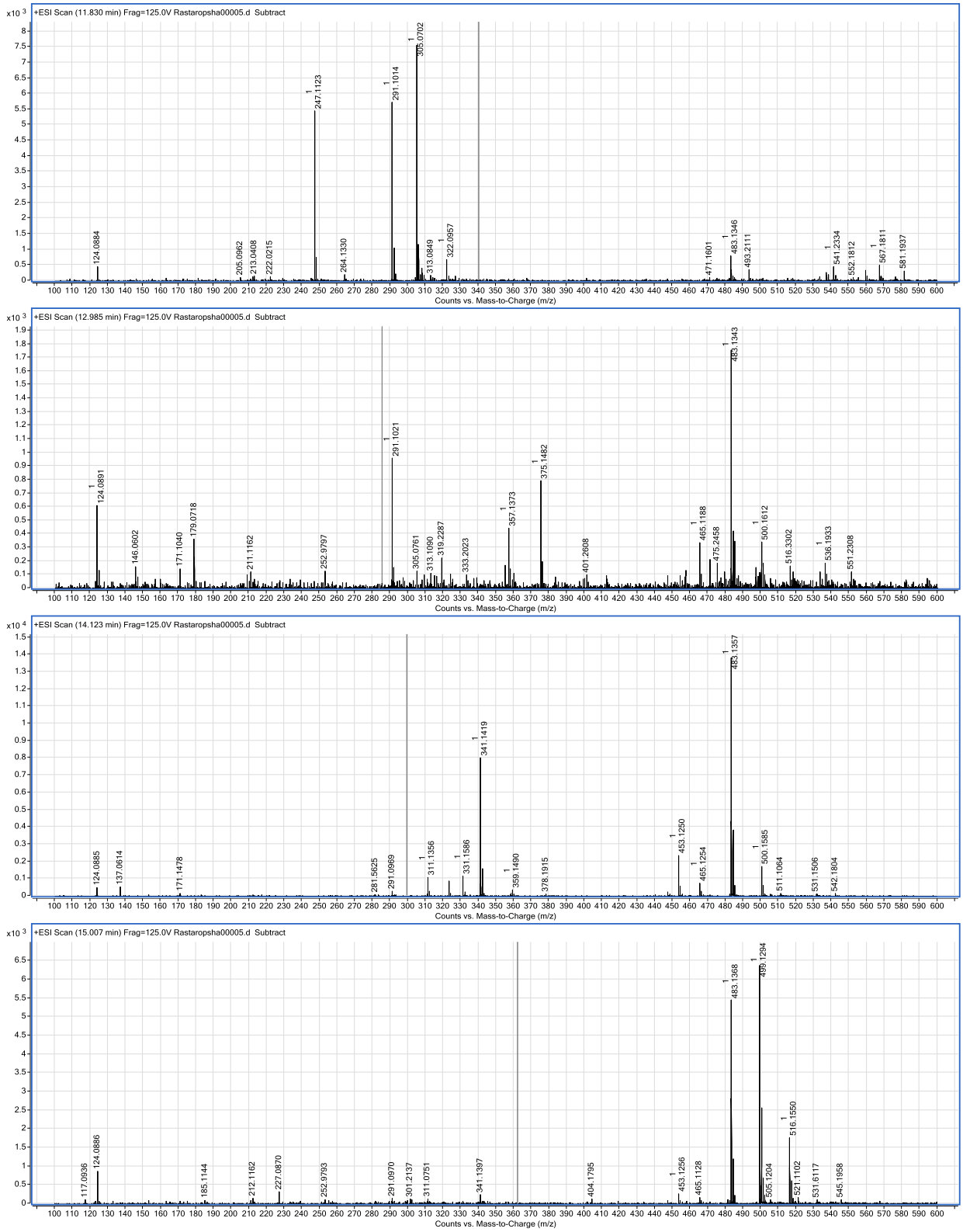


Fig. 3.2. Initial and final indicators of standard extract analysis results
Comparison with standard extract: helps to compare and contrast the results.

Comparative analysis, on the other hand, increases the efficiency and reliability of results. The main final figures are given in Table 3.1.

Now we continue the study (in mass spectrometry) with the extract of the seeds collected from our environment (fig.3.3). Its position over time is given in fig.2.3. The main result units obtained from it are described in Table 3.2.



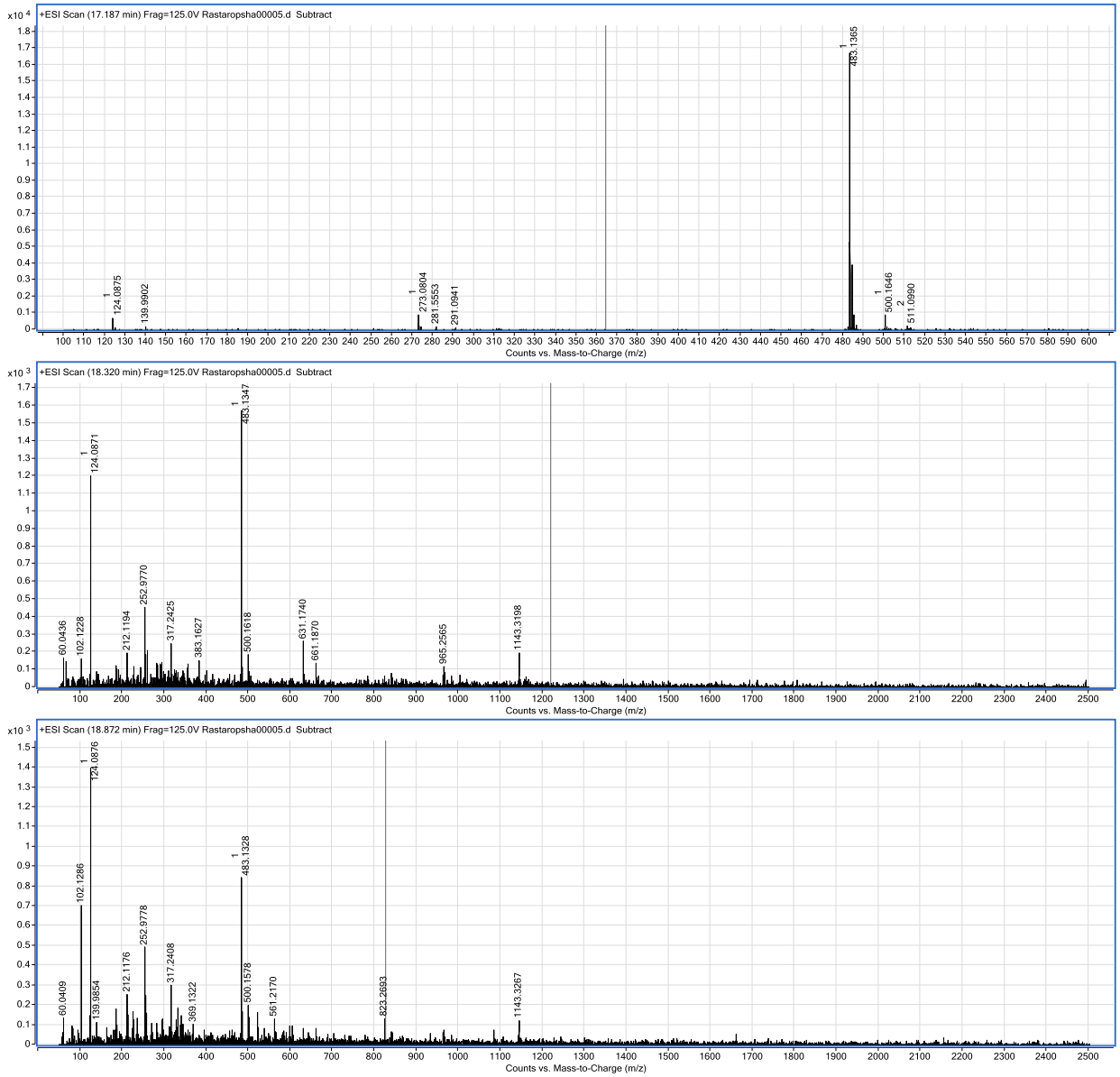


Fig. 3.3. Analysis of the extract of *S. Marianum* seeds in the environment of Uzbekistan in mass spectrometry prepared in our laboratory

№	Retention time-min	Substance name	Mass
1	11.752	Taxifolin	305.0689
2	12.842	isosilychristin	483.1341
3	13.449	Silychristin A	483.1347
4	14.257	Silydianin	483.1363
5	14.989	Silychristin B	483.1362
6	16.253	Silybin A	483.1366
7	16.524	Silybin B	483.1346
8	17.294	Isosilybin A	483.1345
9	17.684	Isosilybin B	483.1360

Table. 3.1. Basic outcomes of standard extract analysis.

№	Retention time-min	Substance name	Mass
1	11.830	Taxifolin	305.0702
2	12.985	Isosilychristin	483.1343
3	13.272	Silychristin A	483.1343
4	14.123	Silydianin	483.1357
5	15.007	Silychristin B	483.1368
6	16.656	Silybin A	483.1361
7	17.187	Silybin B	483.1365
8	18.320	Isosilybin A	483.1347
9	18.872	Isosilybin B	483.1328

Table. 3.2. Indicative values of the analysis in the mass spectrometry of the extract of *S. Marianum* seeds prepared in our laboratory under the conditions of Uzbekistan.

4. CONCLUSIONS

In this study, we studied the number of flavonolignans in the cultivated *Silybum marianum* plant, which is a medicinal wild and at the same time rich in chemical composition, by seed extraction. In the era of chemical technology, the pharmaceutical industry received a lot of attention, and the main reason for this is the human factor. Man needs today's rich chemical composition and raw materials of practical importance. The content of active flavonolignans in *S. Marianum* is incomparably different from other plants. It is this content that can be used as a resource for explicit display, the study of the concentration effects of the content on the body, incorporation into pharmaceutical enterprise methods, and study.

5. ACKNOWLEDGEMENT

The study was conducted at the Sodikov Research Institute of Bioorganic Chemistry, Laboratory of Chemistry of Proteins and Peptides.

6. REFERENCES

[1] Alikaridis, F., Papadakis, D., Pantelia, K., & Kephalas, T. (2000). Flavonolignan production from Silybum marianum transformed and untransformed root cultures. Fitoterapia, 71(4), 379–384. doi:10.1016/S0367-326X(00)00134-9

[2] AbouZid SF, Chen SN, Pauli GF. Silymarin content in Silybum marianum populations growing in Egypt. Ind Crops Prod. 2016 May;83:729-737. doi: 10.1016/j.indcrop.2015.12.012. Epub 2016 Jan 5. PMID: 27182123; PMCID: PMC4863705.

[3] Brantley, S. J., Graf, T. N., Oberlies, N. H., & Paine, M. F. (2013). A systematic approach to evaluate herb-drug interaction mechanisms: investigation of milk thistle extracts and eight isolated constituents as CYP3A inhibitors. Drug metabolism and disposition: the biological fate of chemicals, 41(9), 1662–1670. https://doi.org/10.1124/dmd.113.052563

[4] Sobirov, B. DEVELOPMENT OF DEMAND ON TOURISM SERVICES OVER INNOVATIVE ECONOMIC ADVANCEMENT CONDITIONS EXAMPLES OF UZBEKISTAN. *Journal of Management Value & Ethics*, *85*.

[5] Cardona, M. L., Garcia, B., Pedro, J. R., & Sinisterra, J. F. (1990). Flavonoids, flavonolignans and a phenylpropanoid from Onopordon corymbosum. Phytochemistry, 29(2), 629–631. doi:10.1016/0031-9422(90)85131-X
[6] Kikuchi Y, Miyaichi Y, Tomimori T. [Total synthesis of flavonolignans, scutellaprostins A, B, C, D, E and F]. Yakugaku Zasshi. 1991 Aug;111(8):424-35. Japanese. doi: 10.1248/yakushi1947.111.8_424. PMID: 1665511.
[7] Usmanova Dilafruz Karshievna, Bakaev Ziyovuddinkhon, Safarov Bahodirhon Shaxriyorovich, Sobirov Bobur Baxtishodovich. (2020). Creating free tourism zones as a major feature of the economic development of a region. Evidences from Uzbekistan. *International Journal of Advanced Science and Technology*, *29*(8s), 2274 - 2281. Retrieved from http://sersc.org/journals/index.php/IJAST/article/view/14666
[8] Kuki, Á., Nagy, L., Deák, G., Nagy, M., Zsuga, M., & Kéki, S. (2011). Identification of Silymarin Constituents: An Improved HPLC–MS Method. Chromatographia, 75(3-4),175–180. doi:10.1007/s10337-011-2163-7 (https://doi.org/10.1007/s10337-011-2163-7)
[9] Kim, N. C., Graf, T. N., Sparacino, C. M., Wani, M. C., & Wall, M. E. (2003). Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum). Organic & biomolecular chemistry, 1(10), 1684-1689.
[10] Morazzoni, P., Bombardelli, E., 1995. Silybum marianum (Carduus marianus). Fitoterapia 66, 3–42
[11] National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 6438705. Retrieved October 18, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/6438705.
[12] Polyak, S.J., Morishima, C., Shuhart, M.C., Wang, C.C., Liu, Y., Lee, D.Y., 2007. Inhibition of T-cell inflammatory cytokines, hepatocyte NF-kB signaling, and HCV infection by standardized silymarin. Gastroenterology 132, 1925–1936
[13] Quaglia MG, Bossù E, Donati E, Mazzanti G, Brandt A. Determination of silymarine in the extract from the dried silybum marianum fruits by high performance liquid chromatography and capillary electrophoresis. J Pharm Biomed Anal. 1999 Mar;19(3-4):435-42. doi:10.1016/s0731-7085(98)00231-3. PMID: 10704109.
[14] Scott J. Brantley, Nicholas H. Oberlies, David J. Kroll and Mary F. Paine. Two Flavonolignans from Milk Thistle (Silybum marianum) Inhibit CYP2C9-Mediated Warfarin Metabolism at Clinically Achievable Concentrations. Journal of Pharmacology and Experimental Therapeutics March 1, 2010, 332 (3) 1081-1087; DOI: https://doi.org/10.1124/jpet.109.161927
[15] Abdurazakova, Z. L., Kurbanbayev, I. D., Yunuskhanov, S., Abdushukurova, S., Narimanov, A. A., & Azimov, A. (2020). CHLOROPHYLL CONTENT in the LEAVES of SOYBEAN PLANTS in VARIOUS PHASES of THEIR DEVELOPMENT. Journal of Critical Reviews, 7(13), 173–176. https://doi.org/10.31838/jcr.07.13.30
[16] Jaloliddin Shavkiev, Saidgani Nabiev, Abdulahad Azimov, Shukhrat Khamdullaev, Bakhtiyor Amanov, Hilola Matniyazova, KHushnud Nurmetov. (2020). CORRELATION COEFFICIENTS BETWEEN PHYSIOLOGY, BIOCHEMISTRY, COMMON ECONOMIC TRAITS AND YIELD OF COTTON CULTIVARS UNDER FULL AND DEFICIT IRRIGATED CONDITIONS. Journal of Critical Reviews 7 (4), 131-136. scopus, 1 dona
[17] Азимов, А.А. (2019). Сопряженность вилтоустойчивости с признаком «количество коробочек на растении» у межвидовых гибридов хлопчатника. Международный научно-исследовательский журнал Евразийский Союз ученых 67 (10). 9-13-с. google scholar 1 dona

[18] Azimov, A.A. (2018). To the Genetics of the Wilt-Resistant of Cotton. International Journal of Science and Research (IJSR) 7 (9), pp.1494-1497. google scholarga 1 dona
[19] Abdujjaborovich, A.A. (2018). Verticillium wilt resistance of cotton plants on the basis of interspecific hybridization. European science review (9-10-1)
[20] Abdurazakova, Z. L., Kurbanbayev, I. D., Yunuskhanov, S., Abdushukurova, S., Narimanov, A. A., & Azimov, A. (2020). CHLOROPHYLL CONTENT in the LEAVES of SOYBEAN PLANTS in VARIOUS PHASES of THEIR DEVELOPMENT. Journal of Critical Reviews, 7(13), 173–176. https://doi.org/10.31838/jcr.07.13.30
[21] Tůmová L, Řimáková J, Tůma J, Dušek J (2006). "Silybum marianum in vitro-flavonolignan production" Plant, Soil and Environment. 52 (10): 454–8. 2011-07
[22] 18. Tyagi, A.K., Bhatia, N., Condon, M.S., Bosland, M.C., Agarwal, C., Agarwal, R., 2002. Antiproliferative and apoptotic effects of silibinin in rat prostate cancer cells. Prostate 53, 211–217
[23] Usmanova Dilafruz Karshievna, Bakaev Ziyovuddinkhon, Safarov Bahodirhon Shaxriyorovich, Sobirov Bobur Baxtishodovich. (2020). Creating free tourism zones as a major feature of the economic development of a region. Evidences from Uzbekistan. *International Journal of Advanced Science and Technology*, *29*(8s), 2274 - 2281. Retrieved from http://sersc.org/journals/index.php/IJAST/article/view/14666
[24] Wenzig, Eva; Kunert, Olaf; Ferreira, Daneel; Schmid, Martin; Schühly, Wolfgang; Bauer, Rudolf; Hiermann, Alois (2005). "Flavonolignans fromAvenasativa". Journal of Natural Products. 68 (2): 289–92. doi:10.1021/np049636k
[25] https://lex.uz/docs/-4785256