



Determination of Physiochemical Properties, Amino Acids, and Other Bioactive Compounds of *Silybum Marianum* Seeds (*Silybum Marianum L.*)

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ABSTRACT

Silymarin extracted from *Silybum marianum* seeds is considered the main active component in *Silybum marianum*. Furthermore, multiple pre-clinical investigations have indicated that its major active component silybin has anti-tumoral action in several cancer cell lines, as it may cause growth inhibition and apoptosis and can improve the therapeutic potential of doxorubicin, cisplatin, and carboplatin. The majority of silybin's anticancer efficacy has been identified through in vitro research in tumor cells. In vitro and in vivo cells have revealed the efficacy of silymarin in the prevention and treatment of liver disorders and primary liver cancer. Remarkably high anti-tumor promoting activity and many other pharmacological activities have also been reported, Silymarin extract contains approximately 65% to 80% flavonolignans the chemical structures of silymarin indicated that silymarin is composed of a mixture of (8) major flavonolignans including (silychristins A and B, silydianin, silybins A and B and isosilybins A and B , Also, 2,3-cis-silybin A and B have also been reported, Flavonoids represent 0.58% , while, Phenols 1.15% and lipid 20.2%.

KEYWORDS: Hepatoprotection, herbal drugs, milk thistle, silybin and silymarin, HPLC Analysis

1. INTRODUCTION

It is an annual or biennial plant that blooms in July and August, grows in warm, dry soil, and its heights may reach up to 1.8 meters. It has reddish-purple blooms. (Bijak et al., 2017).

Native origins are Southern Europe, Southern Russia, Asia Minor, and Northern Africa, adapted in North and South America, and South Australia, Also, Now it's grown commercially as a medicinal plant in Europe, Egypt, China, and Argentina (Veres et al., 2012).

The primary active ingredient in milk thistle fruits is silybin, also known as silibinin, which is a combination of flavonolignans referred to as silymarin. In addition to being anti-inflammatory, immunomodulating, antifibrotic, antioxidant, and cardioprotective against cisplatin and doxorubicin toxicity the heart from myocardial damage. silymarin's effects on liver diseases are extensive, encompassing patients with alcoholic liver disease as well as those with nonalcoholic fatty liver disease, viral hepatitis, drug-induced liver injury, and mushroom poisoning (Abenavoli et al., 2018). the extracted Silymarin contains about 65% to 80% flavonolignans (Surai et al., 2015).

Milk thistle seeds are a rich source of antioxidants and food preservatives because of their exceptional free radical scavenging activity and ability to prevent lipid peroxidation. Scholars have also suggested that the seeds may have potential positive health effects and present opportunities for the development of value-added products. (Serçeand et al., 2015).

According to research, *Silybum marianum* influences the formation of galactose-induced cataracts in rats. The group of cataracts treated with silymarin showed a considerably delayed development compared to the control group. When silymarin was administered to rats fed galactose, it prevented the development of cataracts and strengthened the antioxidative defence system by increasing GSH and lowering LPO levels in the lenses of the treated rats, as compared to the control group (Huseini et al., 2009).

Silymarin has low solubility in water of less than (50 µg/mL), poor bioavailability and absorption of about (23–47%) and reaches systemic circulation after oral administration. (Ayaz et al., 2018), (Bijak et al., 2017).

The mixture of (silymarin, boswellic acid, and curcumin) has anti-carcinogenetic properties, whether “in vitro” or “in vivo” and may prevent inherited intestinal cancer in an animal model. (Girardi et al., 2020).

Silymarin has been shown to have a significant effect on prostate and breast cancer by controlling cell division and DNA synthesis, as well as fighting against viral infection, inflammation, and cytotoxicity. It also has a hypocholesterolemia

effect and hypertensive activity by reducing heartbeat, systolic basal and arterial blood pressure in culture and nerve growth factor-prompted neurite outgrowth in cervical cancer. (Zahra et al ., 2017).

2. MATERIALS AND METHODS

2.1. Materials

Silybum marianum seeds were purchased from “Wadi El Shah farm -armed forces” All chemicals used in these experiments were purchased from “The El Nasr company “ for the pharmaceutical industry with high quality and purity.

2.2. Analytical methods

Moisture, ash, fiber, total flavonoid, total phenols, and total antioxidant (dpph): were determined according to the method of the Association of Official Analytical Chemists (A.O.A.C. 1980).

2.2.1. Total carbohydrates were estimated.

According to (Dobois et al. 1956).

Plant matter was acid hydrolyzed in a sealed tube with an accurate known sample (0.2 g) and 10.0 ml of 1.0 M H₂SO₄ solution. Then boiled for 10 hours. neutralized with barium carbonate, and the precipitate was removed by filtering the solution with No.1 filter paper using, phenol-sulfuric 1.0 ml of acid hydrolysate + 5.0 ml of H₂SO₄ + 1.0 ml of phenol solution (5%) were added.

The formation of the yellow-orange color was estimated through a spectrophotometer at 490 nm.

2.2.2. Crude protein was estimated :

According to (AOAC.1980)

Determination of proteins was conducted by using the micro-Kjeldahl technique multiplying the values of total nitrogen with a 6.25 factor.

2.2.3. Crude lipid was estimated:

According to (A.O.A.C. 1990)

Using the Soxhlet technique to perform this estimate.

A gram of sample was weighed and filtered then lipid was extracted using 200 ml of n-hexane.

2.2.4. Determination of total phenolic content (TPC)

As described by Simons and Ross (1971) using the Folin-Ciocalteu reagent.

The reagent solution containing (4% Na₂CO₃ (in NaOH 0.1 M), 0.02% potassium sodium tartrate tetrahydrate, and 0.02% CuSO₄) in a volume of 180 µL +10 µL sample. After 10 minutes at room temperature, the Follin reagent was added 10 µL and measured at 735 nm with a spectrophotometer.

Total phenolic production (mg•L⁻¹) = DW (g•L⁻¹) × TPC (mg•g⁻¹)

2.2.5. Determination of Total flavonoid content

A 1 ml of extract + 4 ml of distilled water + 0.30 ml of (5% sodium nitrite was added to the flask, and after 5 minutes, 0.3 ml of 10% aluminum chloride was mixed.

Using a UV/Visible spectrophotometer to measure the absorbance at 510 nm.

The flavonoid content concentration was represented in milligrams of QE per gram of extract.

The total flavonoid content was expressed as mg of QE/g of extract.

2.2.6. Total Antioxidant Activity DPPH

2.2.6.1. Sample preparation

The supplied plant material (523.3mg) was sonicated for 30 minutes and macerated for one day before filtering. The extract was then collected and dried under vacuum at 50°C, yielding 4.8 mg of orange residue. In ethanol, samples were produced at 750, 1000, 1250, 1500, 1750, and 2000 g/mL final concentrations.

2.2.6.2. Trolox standard

A standard solution of 20 µg/mL concentration of Trolox was prepared in methanol from which 6 concentrations were prepared including 10, 7.5, 6.25, 5, 3.75, and 2.5µg/mL.

2.2.6.3. Procedure

Described by Boly et al 2016.

100L of sample + DPPH reagent (0.1% in methanol) was mixed in a then incubated at room temperature for 30 minutes in the dark. The intensity of the color was measured at 540 nm.

The following equation is used for % inhibition: (Average absorbance of blank-average absorbance of the test)/(Average absorbance of blank) *100.

2.2.6.4. Data analysis

The data was processed in Microsoft Excel®, and the IC₅₀ value was computed in Graph Pad Prism 6® by converting the concentrations to logarithmic values and choosing a non-linear inhibitor regression equation (log (inhibitor) vs. normalized response - variable slope equation).

2.2.7. Determination of Amino acids

Sykam Amino Acid Analyzer (Sykam GmbH, Germany) was used.

(Quaternary pump with flow range 0.01 to 10.00 ml/min and maximum pressure up to 400 bar) with built-in dual filter photometer between 440 and 570 nm with constant.

2.2.7.1. Sample preparation

5 mL of hexane was combined with 300 mg of the sample. For a whole day, the mixture was left to macerate. After filtering the mixture using Whatman No. 1 filter paper, the residue was put into a test tube and heated to 110°C for 24 hours in an oven. The sample was filtered using Whatman No. 1 filter paper after incubation, evaporated on a rotary evaporator, and dissolved in 100 ml of dilution buffer. One milliliter was then diluted in a 3-milliliter vial, filtered through a 0.22 µm syringe filter, and 100 µl was injected.

2.2.7.2. Instrument parameters

Column: LCA K06/Na
Mobile phase: Buffer A, Buffer B, and Regeneration solution.
Mode of elution: Gradient
Flow rate: 0.45 ml/min
Temperature: Gradient 57C-74C
Wavelength: 440 and 570 nm
Buffers and solutions preparation

Parameter	Buffer A	Buffer B	Column Regeneration solution	Sample dilution buffer
pH Value	3.45	10.85	----	2.20
Normality	0.12	0.20	0.50	0.12
Trisodium citrate dihydrate	11.8 g	19.6 g	----	11.8 g
NaOH	----	3.1 g	20.0 g	----
Citric acid	6.0 g	----	----	6.0 g
Boric acid	----	5.0 g	----	----
Methanol	65 ml	----	----	----
Thiodiglycol	----	----	----	14 ml
Hydrochloric acid 32%	6.5 ml	----	----	12 ml
EDTA	----	----	0.2 g	----
Phenol	0.5 g	----	----	2.0 g
Final volume	1 L	1 L	1 L	1 L

2.2.8. Determination of HPLC Analysis

Before the injection (10 μ l), the sample was extracted using 80% methanol and then filtered through a 0.45 μ m micropore membrane.

The sample was subjected to analysis at a flow rate of 0.7 milliliters per minute using an Aligent 1200 LC-MS –ESI apparatus (positive mode) equipped with a diode array detector set at 280 nm and an Aligent zorbax eclipse plus C18 column, which was nebulized with nitrogen.

One percent formic acid (A) and acetonitrile (B) were utilized as the mobile phase. The gradient was 0 min 10% B, 10 min 40% B, 20 min 80% B, 2anned 5 min 90% B, and 30 min 10% B.

3. RESULT

Table 1. Chemical composition of *Silybum marinum* seeds (g/100g dry weight basis).

constituents	Percentage
Moisture	19.54%
Ash	11.92%
Fiber	16 %
Total phenols	1.15%
Total Flavonoid	0.58%
Total carbohydrate	24%
Total protein	6.73%
Total lipids	20.2%

Total antioxidant activity DPPH

Table 2. Trolox concentration dpph % inhibition

Trolox concentration (μ M)	% inhibition
5	9.14
10	16.08
15	27.07
20	35.84
30	56.27
40	76.07

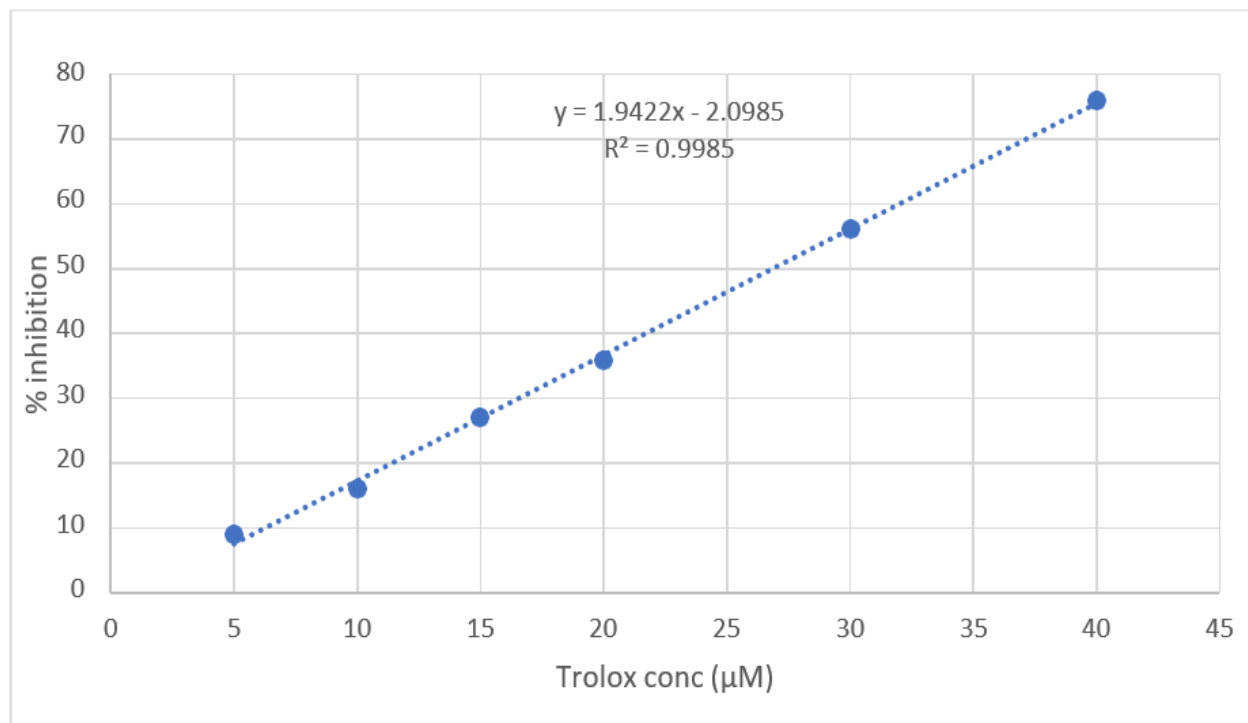


Figure 1. % inhibition and concentration per (µM):

Table 3. % Average of inhibition of silymarin extract

Average % inhibition	Micro molar Trolox equivalent per mg extract (µM TE/mg extract)	Standard deviation
52.227	27.971	1.021

In Silybum. Marianum extract from the DPPH method revealed that the percent of inhibition increased with increasing concentration, where concentration 40 µM/mg yield was 76.07% inhibition while concentration 5

µM/mg yield was 9.14%. The relationship between the concentration and percent of inhibition was plotted to determine IC50 (50 % inhibitory concentration) where IC50 was found to be 52.227. (Table 3) Figure1.

Determination of Amino acid analyzer

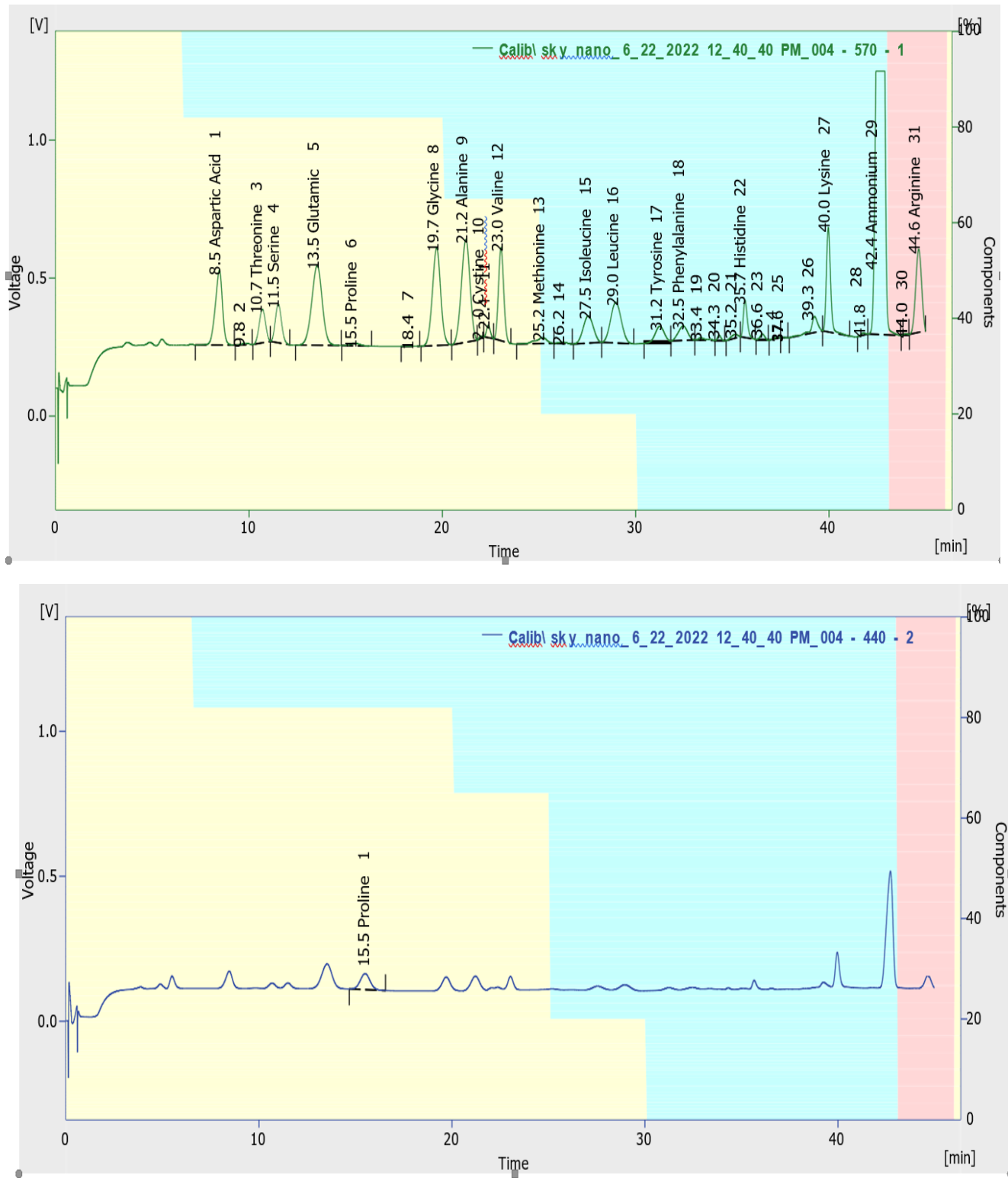


Figure 2.

Table 4.

Result Table (ESTD - Calib\sky normal_6_22_2022 1_43_16 PM_005 - 570 - 1)

	Reten. Time [min]	Area [mV.s]	Amount [mg/100mg]	Compound Name
1	8.440	10402.628	0.289	Aspartic Acid
3	10.629	3725.424	0.119	Threonine
4	11.456	3970.075	0.106	Serine
5	13.507	8275.317	0.262	Glutamic
6	15.493	462.762	0.325	Proline
8	19.805	14339.305	0.233	Glycine
9	21.285	16687.349	0.458	Alanine
10	22.328	934.689	0.050	Cystine
11	22.981	9310.672	0.230	Valine
12	25.136	848.460	0.026	Methionine
14	27.488	5156.243	0.158	Isoleucine
15	28.907	7701.443	0.233	Leucine

Result Table (ESTD - Calib\sky normal_6_22_2022 1_43_16 PM_005 - 570 - 1)

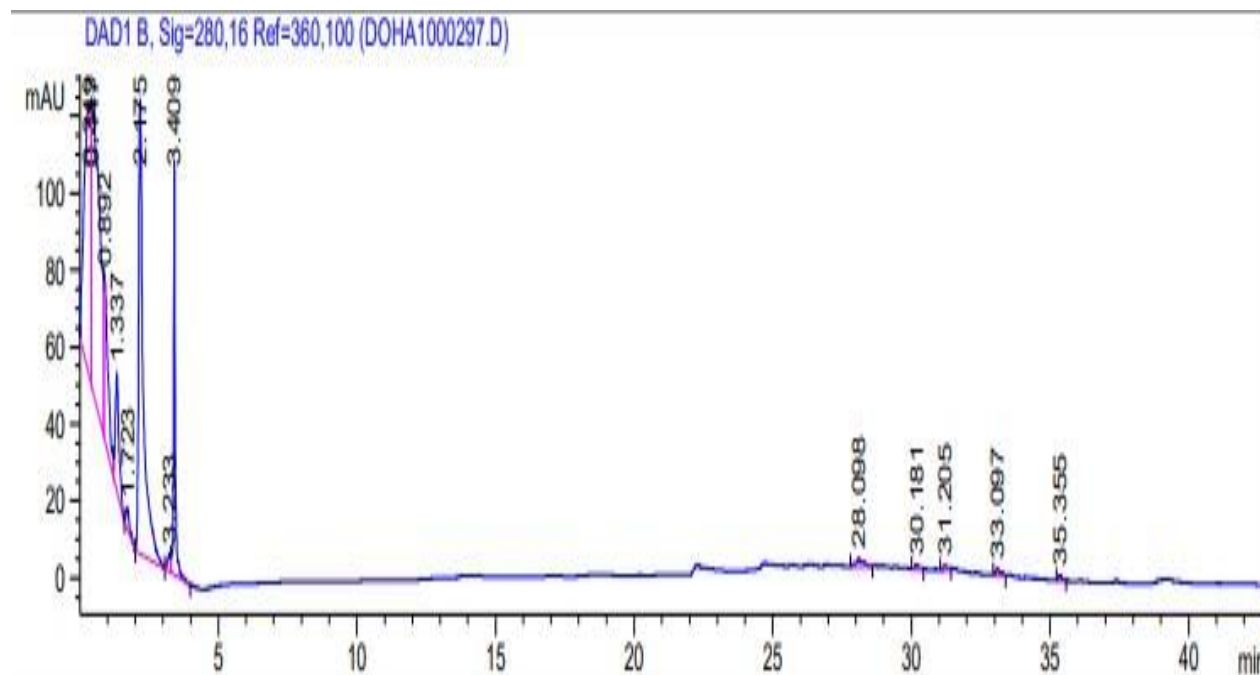


	Reten. Time [min]	Area [mV.s]	Amount [mg/100mg]	Compound Name
16	31.181	2647.856	0.112	Tyrosine
17	32.379	2488.293	0.098	Phenylalanine
21	35.563	2777.281	0.088	Histidine
27	39.907	5725.130	0.167	Lysine
29	42.355	36403.568	0.000	Ammonium
31	44.560	8567.627	22.358	Arginine
	Total	140424.121	25.313	

Result Table (ESTD - Calib\sky normal_6_22_2022 1_43_16 PM_005 - 440 - 2)

	Reten. Time [min]	Area [mV.s]	Amount [mg/100mg]	Compound Name
1	15.493	3122.556	18.714	Proline
	Total	3122.556	18.714	

HPLC Analysis



Chromatogram 1. HPLC Analysis Profile of silymarin ethanolic extract (at 0.447min gave the following spectrum Fragment at m/z 228 is due to A1or B2 – 2OH).

Real-time	Phenolic compound	References
0.449	Unknown	
0.892	Silychristin A	
1.337	Silydianin	
1.327	Silychristin B	
1.723	Silybin A	
2.175	Silybin B	
3.409	Silybin isomer	
3.223	Silybin isomer	
28.098	Isosilybin A	Akos Kuki and et al ., (2012).
30.181	Isosilybin B	
31.205	Isosilybin isomer	NOHAIRH. SHERI and et al ., (2017).
33.097	Unknown	
35.355	Unknown	

4. DISCUSSION

Chemical composition of *Silybum marianum* seeds

The chemical composition of *Silybum marianum* seeds was determined and reported in Table (1) has the most concentrated silymarin more than other parts of the plant.

Flavonoids represent the major component of *Silybum marianum* seeds and represent (on a dry weight basis) whereas carbohydrates represent 24%, lipids 20 %, protein 6,73 % and phenols 1,15 %. The results showed that protein content ranged from 11.81 to 22.31g/100g on a dry weight basis, The accomplished results on the other hand, are in

agreement with those reported by (Barreto et al., 2003).

(Michael et al., 2017) reported that total carbohydrate concentration in the seed ranged from 24.2 to 26.3 % , while total lipids varied from 19.74% to 23,19 % and total flavonoid from 16.01 % to 29.09 % obtained by (Aziz et al., 2020).

The acquired results of the amino acid analyzer illustrated the percentage of composition of the dry seed extract of milk thistle is (9) essential and (9) nonessential amino acids. In particular, isoleucine, histidine, threonine, methionine, tryptophan, valine, lysine, phenylalanine, and leucine are more abundant than other amino acids. The amount of leucine isoleucine and histidine was much lower, and (9) nonessential amino acid includes arginine , alanine ,cysteine, asparagine , aspartic , tyrosine , proline , glycine ,glutamic which corresponds to a hundred measurements.

All information about the amino acid analyzer contained in this article is purchased by Nawah Scientific Center.

Table (5): shows the phenolic compound of SM seeds that separated in positive mode at 0.447min gave the following spectrum Fragment at m/z 228 is due to A1 or B2 – 2OH and the major compound was silymarin which consists of (9) flavolignin (Silychristin A- Silydianin-Silychristin B- Silybin A- Silybin B- Silybin isomer- Isosilybin A- Isosilybin B- Isosilybin isomer) that responsible for the antioxidant scavenging activity, due to stopping free radical formation by inhibiting specific ROS-producing enzymes, decrease localized oxygen concentration and preventing the effect of various chemicals such as (alcohols, carbon tetrachloride, cisplatin, doxorubicin, Heavy metals, gamma radiation, aflatoxin) and preventing DNA damage through several mechanisms.

Based on the above results *Silybum marianum* seeds are an interesting plant with a variety of uses. Because of the chemical composition of their seeds and the limits of phenolic and flavonoid compounds that reach to phenols 1,15 %,0.58%.

5. CONCLUSION

In conclusion, the current study supports the presence of several significant phytochemical compounds in the seeds of *Silybum marianum*.

The use of seed extracts from *Silybum marianum* as a natural agent to protect against peroxidative damage in biological systems connected to inflammation, aging, and carcinogenesis should be further researched.

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الملخص العربي

تقدير الخواص الكيميائية والفيزيائية والاحماض الامينية والمركبات الاخري الفعاله لبذور نبات شوك الجمل

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تم تقدير التركيب الكيميائي لبذور نبات شوك الجمل والذي يعد من الأعشاب الطبية القديمة، وهي تنتمي إلى العائلة النجمية، لها العديد من الأسماء، مثل: خرشوف الجبل، والأرضي الشوكي البري، والعكوب، وهي نبتة تنمو في أوروبا وآسيا وشمال إفريقيا، عشبة شوكة الجمل من أبرز النباتات التي تساعد على علاج الكثير من الأمراض، لاحتوائها على مضادات الأكسدة أو مخلوط الفلافونيدات وأبرزها مركب السيلمارين الذي يساهم في تعزيز الجهاز المناعي للجسم؛ - عشبة شوكة الجمل من أبرز النباتات الطبية التي تعمل على مكافحة العديد من البكتريا والطفيليات المسببة لمعظم الأمراض تم التقدير على أساس الوزن الجاف فكان المحتوى من الرطوبة ١٩,٥٤%، والرماد ١١,٩٢%، والفينولات ١,١٥%، الفلافونيدات الكلية ٠,٥٨%، والكربوهيدرات ٢٤%، والروتينات ٦,٧٣%، والليبيدات ٢٠,٢% ومضادات الأكسدة ٢٦٤٥,٠ ملليج / ١٠٠ ملجم مقدرة كحامض الأسكوربيك وتم تقدير الأحماض الأمينية في البذور وكانت (أرجنين - ألانين - سيستائين - أسباراجين - أسبارتك - تيروزين - برولين - جليسين - جلوتاميك - ميثونين - هستدين - أيزوليوسين - فينيل الانين - فالين - ليسين) وتم التعرف على المركبات التي لها نشاط مضاد للأكسدة من خلال التحليل الكروماتوغرافي وكانت كالأتي :

(Silybin A and B – Silychristin A and B – isosilybin A and B – silydianin)

الكلمات المفتاحية : سيلمارين - شوك الجمل - فلافونيدات - سليلين - مواد مضادة للأكسدة .