Chemical Profiling, And Biological Investigation of (Silybum Marianum L.) Seed in Iraq

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Abstract
The main objective of the current study was to evaluate the Chemical Profiling, and Biological Investigation of (Silybum Marianum L.) Seed in Iraq, Sylibum marianum seeds were purchased from the local market in AL-Muthanna and Baghdad. The study was comprised of two phases. In 1st phase, nutritional composition, that is, moisture, Lipid, protein, and ash, was determined according to their respective methods. Moreover, The identification of free amino acids, Fatty acids, and individual phenolic compounds was performed by an amino acid analyzer, Gas chromatography GC, and High-Performance Liquid chromatography (HPLC) respectively in 2nd phase. The result Silybum Marianum seeds are rich in Carbohydrate, protein, and lipids content which was 54.732 g/100 g, 27.133 g/100 g, and 20.066 gL100g respectively. Silybum Marianum contains five fatty acids, Thirteen amino acids, five phenolic compounds, Ferulic acid, Gallic acid, Rutin, kaempferol, Qurcetine were 96.766, 86, 66, 13.1, 41.066 µg/gm identified as the major phenolic compound respectively. The present study indicated that seeds of S. marianum have phenolic contents. Therefore, Silybum marianum can be used as a potentially rich source of antioxidants and food preservatives. The results suggested that seeds from S. marianum might have potential health applications and provide opportunities to develop value-added products, suggesting that seeds of S. marianum would be useful for health by acting as bifunctional.
Keywords: Silybum marianum, Chemical Profiling, Biological activity

1. Introduction

Medicinal plants play an important role in human health care. About 80 % of the world's population relies on the use of traditional medicine, which is mainly based on plant materials [1]. Recently, various medicinal plants and their phytoextracts have shown numerous medicinal properties like antioxidant, anti-inflammation, anti-cancer, anti-microbial, anti-diabetes, anti-nociceptive action, etc.[2]. In India, about 2500 plants have been reported to be used in ethno- medicine[3].
Silybum marianum, commonly known as (milk thistle), belonging to Asteraceae family is an annual or biennial herb, native to the Mediterranean and North African regions, but now widespread throughout the world [4]. The principal active constituents of milk thistle is an isomeric mixture of flavonolignans, the main components of silymarin are silybin, isosilybin, silychristin and silydianin . Silymarin localized mainly in the external cover of the seeds (1.5-3 %). Other constituents are essential fatty acids, flavonoids e.g. taxifolin, kaempferol, and quercetin [5]. It is being used as a general medicinal herb from as early as 4th century B.C. and first reported by Theophrastus[6]. Silymarin is mainly in the seed shell and seed kernel contains mainly protein and oil [7]. The oil contains a relatively high content of vitamin E and a great quantity of the unsaturated fatty acids such as linoleic (C18:2) and oleic acid (C18:1) [8]. The protein (mainly albumin) in seed kernel is also very nutritional in essential amino acids, and the processing properties of the protein were excellent. The solubility of the protein was better and its foaming capacity and foam stability, emulsification capacity and stability were remarkably superior to that of the SPI [9]. Thus, the protein from seed kernel has good potential to be applied as a valuable source of protein nutrition. The aim of this study is to investigate the proximate composition, phytoconstituents (estimate the phenolic compounds and fatty acids, and amino acids) of (Silybum Marianum L.) Seed in Iraq.

2. Materials and Methods

2.1 Chemical materials

Sulfuric acid, Sodium hydroxide, ethanol, methanol, formic acid, hydrochloric acid, OPA (ortho phethalene aldehyde), distilled water

2.2 Methods

2.2.1 Plant collection and plant sample preparation

Sylibum marianum seeds were purchased from the local market in Muthanna and Baghdad in the winter of December 2021, and the seed was transported to the laboratory. After that, The seeds were clean, washed, and dried in an oven at (40 °C) The dried seeds were then ground to powder. The seeds powder was then kept in sealed plastic bags and stored at – 20 °C until further analysis.

2.2.2 Proximate Analysis of *Sylibum marianum* seeds

Determination of the chemical compositions of the dates was in accordance with the procedure of the Association of Official Analytical Chemists [10]. This includes moisture (method 934.06), protein by Kjeldahl nitrogen (method 920.152), crude fiber by

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acid, alkaline digestion methods using 20% H2SO4and 20% NaOH solutions (AOAC, 2006), and ash determination (method 940.26). The content of crude protein was determined by the multiplication of total nitrogen content with a factor of 6.25 [10]. Bligh and Dyer's procedure [11], was employed in the determination of lipid content. Total carbohydrates were calculated by subtracting the total percentage value of other measurements from 100. Proximate analyses were expressed as grams per 100 g of fresh weight.

2.2.3 Extract of Phenolic Compounds in Sylibum marianum

The phenolic compounds were extracted from the homogenized plant sample (3g) using ethanol/water (70/30) solvent. The extraction process was carried out using Ultrasonic Bath
(USA) at room temperature for 1 hour [12]. After filtration, 5 mL of liquid extract was used for extraction yield determination. The solvent was removed by rotary evaporator under vacuum (Slovenia) and was dried at 40°C to the constant mass. Dry extracts were stored in the glass bottles at 4°C to prevent oxidative damage until analysis. Quantification of individual phenolic compounds was performed by reversed-phase HPLC analysis, model SYKAMN (Germany) chromatographic system equipped with a UV detector), the column separation was Chemstation, a Zorbax Eclipse Plus-C18-OSD (25cm, X 4.6mm). The column temperature was 30 °C the gradient elution method, with eluent A (methanol) and eluent B (1% formic acid in water (v/v)) was performed, as follows: initial 0-5 min, 40 % B; 6-15 min, 50 % B; and flow-rate of 1.2 mL/min. The injected volume of samples 100 µL .and standards was 100 µL and it was done automatically using an autosampler. The spectra were acquired at 280 nm [13].

2.2.4 Determination of Fatty acid in *Sylibum marianum* Extract

The fat was estimated based on the [14] (AOAC 1995) method using a fat extraction device (Soxhlet). The sample was prepared according to the method approved by[14] (AOAC 1995). The fatty acid compounds were analyzed using a gas chromatography device (GC - 2010) of the Japanese-origin Shimadzu model, where the ionized flame detector was used and a capillary column type (SE-30) with lengths (30m * 0.25 mm) was used according to the following conditions Injection area temperature 28 °C, Detector temperature 31 °C, Separation column temperature 120-290 (10 C/MIN), and Gas Flow Rate 100 K p a.

2.2.5 Amino Acid Extraction

About 5 mg solid samples were weighed with an accuracy of 0.01 mg and approximately 100 mg liquid samples were weighed with an accuracy of 0.01 mg then 1 ml of 6 M hydrochloric acid solution as hydrolysis agent was added, the tube was covered, and placed in the aluminum thermo block at 100 °C±20 °C for 24 hours for hydrolysis, Using a pipette, a volume of 100 µl of hydrolyzed is introduced in a vial placed in evaporation to remove moisture with nitrogen gas. The dried amino acid residues were dissolved in a volume of 100 µl of acetonitrile. They are derivatives with a volume of 100 µl of OPA (ortho phethalene aldehyde). The sealed vial is subjected to ultrasound for 1 minute. The vial is placed in the thermo block at 100 °C+2 °C for 30 min. To complete the derivatization reaction. The vial is placed in the gas chromatograph sample stand 10 injections of 100 µl per sample are performed [15].

2.3 Statistical Analysis

The recorded data were analyzed by SPSS. Statistical differences between the samples and the controls were evaluated by the test of one-way analysis of variance (ANOVA) and Tukey's post-test analysis was performed to evaluate significant differences among the obtained bioassay data at 95% confidence interval. The outcome of the results was presented as the mean of three replicate experiments ± standard deviation (SD).

3. Results and Discussion

3.1 Proximate composition

Proximate analysis was carried out to see the variation in the composition that can affect The nutrient content and biological activity of the sample. The results showed a high Variation among the studied sample (Table 1).

Table 1: Frequency distribution of sample compounds according to Mean of concentration.

Concentration	Compositions of Samples					P value
	Ash	Moisture	Protein	Lipid	Carbohydrate	
Proximate						
Mean ±SD	2.30 e±0.1	5.233 d± 0.3	27.133b ±0.05	20.066 c ± 0.3	54.732a± 0.2	p≤0.001
Range	2.3 -2.1	5.5 - 4.9	27.8 – 26.9	25.3 -24.7	54.8 – 53.9	
SE	0.057	0.17	0.078	0.176	0.089	
Energy value (Kcal/100g)	387.658					
SD: standard deviation; P: One-way Anova; Different letters referees to a significant at p ≤ 0.001						

Results showed that the highest Carbohydrate, protein, and lipids content which was 54.732 g/100 g, 27.133 g/100 g, and 20.066 gL100g respectively. The moisture contents was 5.233 g/100g. The ash was found 2.30 g/100 g. Previous studies on biochemical profile of milk thistle (Silybum Marianum L.) with special reference to silymarin content for proximate composition

[16]. The findings of our study are in agreement with the results reported by previous studies [17]. The studies reported Silybum Marianum contain adequate amounts of many essential nutrients and the result for proximate agreement with our study. The present study showed that Silybum Marianum contained a high amount of carbohydrate, crude protein, lipids and low amount of ash.

3.2 Phenolic and flavonoids composition

Phenolic and flavonoid compounds are well known for their antioxidant activity which can reduce the production of radicals and lower oxidative stress [18]. The phenolic and flavonoid compounds which are responsible for various biological activities of plants are usually present as micro-constituents of plants [19] . The phenolic compounds level in the ethanolic extracts was measured in accordance with the procedure of Folin-Ciocalteau. The results of the study showed in Table (2). Two phenolic compounds namely, Gallic acid and Ferulic acid were 96.766 and 86 were obtained respectively, The phenolic concentration of our study was higher than those investigated by [20]. In previous studies, by [21]. disagree with our study result.

Table 2: The Mean Concentration of Phenolic compounds in plants.

Compounds (mg/kg)	Mean Concentration			P value
	Mean	SD	SE	
Type of Phenolic compounds				
Gallic acid	96.766a	0.14	0.145	p≤0.001
Ferulic acid	86b	0.05	0.035	

SD: standard deviation; P: One way Anova; Different letters referees to a significant at p ≤ 0.001

The results of the flavonoids showed in Table (3). three flavonoids compounds namely, Rutin, kaempferol, Qurcetine were 66, 13.1, and 41.066 were obtained respectively. Certain flavonoids, particularly quercetin, rutin and kaempferolare known to exhibit significant antioxidant effects as well as anti-diabetic properties. The flavonoids compounds of our study was agreement with those investigated by [22].

Table 3: The Mean Concentration of flavonoids compounds in plants.

Compounds (mg/kg)	Mean Concentration			P value
	Mean	SD	SE	
Type of Flavonoids compounds				
Rutin	66a	0.09	0.015	p≤0.001
kaempferol	13.1c	0.03	0.051	
Qurcetine	41.066b	0.15	0.045	

SD: standard deviation; P: One way Anova; Different letters referees to a significant at p ≤ 0.001

33 Fatty acid content

The fatty acid composition of Silybum marianum seeds was analyzed in their hexane layer forms using Gas Chromatography (GC-2010). Identification and relative percentage quantitative analysis of the fatty acids are shown in Table 4. This study showed the presence of five fatty acids namely, Palmatic acid, Stearic acid, Oleic acid, Linolic acid, and Lenolinic acid were 5.673, 3.24, 18.51, 22.086, and 1.216 % obtained respectively. The composition of the oil revealed that Silybum marianum oil is fairly high in polyunsaturated fatty acids particularly an essential fatty acid i.e., α-linoleic acid (Omega-3) which is about 8.7 ppm, this fatty acid is believed to be helpful in lowering cholesterol when induced in the diet[23]. Previous studies by [24]. reported that S. marianum seed oil has high unsaturated fatty acid content which constitutes (73.0%) of the total fatty acids. Linoleic acid content was 53.30% of the total composition followed by oleic acid (20.80%). Another study reported that the main fatty acids detected in the Iranian S. marianum seeds were: linoleic acid (45.36%) followed by the oleic acid (31.58%). We have also detected the presence of palmitic acid (C16:0), stearic acid (C18:0), Arachidic acid C20:0, γ-linolenic acid C18: 3 (n-6) and Cis-11-Eicosenoic acid C20:1n-9 in S. marianum seeds [25]. It can be said that our results have shown compatibility with the other S. marianum seed essential oil studies, especially on major compounds.

Table 4: The Mean Concentration of Fatty acid compounds in plants.

Compounds%	Mean Concentration			P value
	Mean	SD	SE	
Type of Fatty acid compounds				
Palmatic acid	5.673c	0.047	0.027	p≤0.001
Stearic acid	3.24d	0.034	0.020	
Oleic acid	18.51b	0.045	0.026	
Linolic acid	22.086a	0.032	0.018	
Lenolinic acid	1.216e	0.035	0.020	

SD: standard deviation; P: One-way Anova; Different letters referees to a significant at p ≤ 0.01

3.4 Amino acids content

Silybum marianum contained varying quantities of different types of free amino acids. The amino acid composition of S. marianum seeds is presented in Table 5. The results show Thirteen amino acids, were detected Alanine, Glycine, Valine. Leucine, Serine, Proline, Asparagine, Aspartic Acid, Methionin, Glutamic, Tryptohan, Phenylalanine, and Lysine. Alanine was higher concentrated (59 µg /gm) While Valine was less concentrated (16.033 µg /gm) [26]. stated that milk thistle protein considered a poor source of proline (0.37%) and histidine (1.44%). However, threonine is the higher (16.66%). High amounts of lysine, isolucine, leucine, valine, and threonine,[27]. mentioned that partially defatted milk thistle seeds protein contained markedly amounts of essential amino acids such as arginine (12.59%), leucine (9.84%), valine (7.97%) and lysine (7.38%). All these essential amino acids can be found in plant foods [28]. Figure1. Chromatographic Amino Acid Analyzer of Silybum marianum

Table 5: The Mean Concentration of Amino acid compounds in plants.

Compounds (µg /gm)	Mean Concentration			P value
	Mean	SD	SE	
Type of Amino acid compounds				
Alanine	59[a]	0.4	0.230	p≤0.001
Glycine	36[g]	0.458	0.264	
Valine	16.033[k]	0.321	0.185	
Leucine	35.7[g]	0.360	0.264	
Serine	49.3[c]	0.458	0.234	
Proline	17.933	0.152	0.088	
Asparagine	17.9[k]	0.1	0.057	
Aspartic Acid	24.833[j]	0.305	0.185	
Methionin	54.830[b]	0.231	0.158	
Glutamic	43.067[e]	0.208	0.120	
Tryptohan	22.9[j]	0.173	0.1	
Phenylalanine	37.6[f]	0.264	0.149	
Lysine	47.4[d]	0.152	0.162	

SD: standard deviation; P: One-way Anova; Different letters referees to a significant at p ≤ 0.01

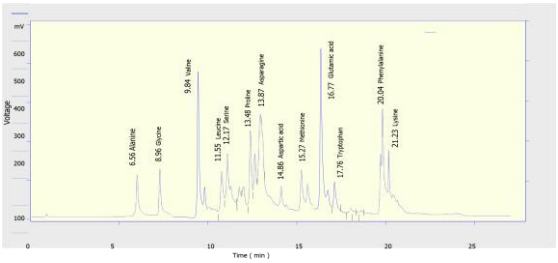

Figure 1: Chromatographic Amino Acid Analyzer of Silybum marianum

4. Conclusion

Silybum marianum is an important medicinal plant and contains high-value active ingredients in the seeds such as fatty acids, phenols, flavonoids, and amino acids. The discovery, characterization, and separation of their bioactive components are critical because these bioactive compounds yield the major clinical benefit of the plant in pathological conditions. Our findings reinforce the potential of Silybum marianum as a valuable source of natural antioxidants and support its medical uses in the treatment of many diseases and suggested the potential use of S. marianum as a functional food

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